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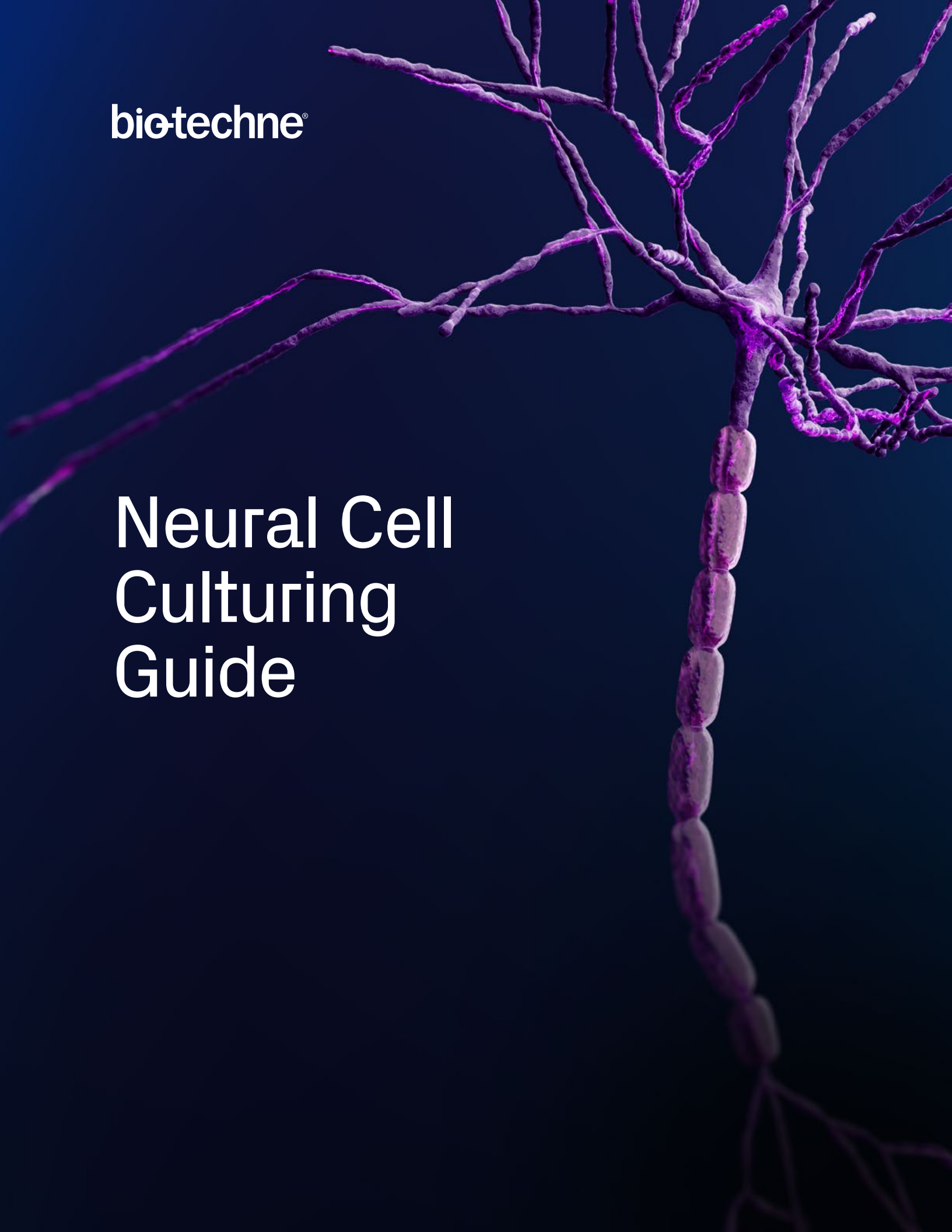
Neural Cell Culturing Guide



The advent of *in vitro* culturing of neural cells has been central to driving our understanding of the nervous system. The complexity of neural tissue makes it difficult to study the cellular and molecular mechanisms underlying neural functioning. Cell culture systems have given researchers the means to study the nervous system at this level. This guide provides an introduction to the techniques and protocols for successfully isolating and culturing specific cell types from the rodent central and peripheral nervous systems, as well as the expansion of mouse and rat cortical stem cells. Contributors include Bio-Techne scientists from our bioassay and stem cell divisions, who have experience in culturing and maintaining multiple types of neural cells. As with every new procedure, these protocols may need to be optimized for your experiment. This guide also presents the industry's premiere media supplements, adhesion substrates, bioactive proteins, verification antibodies, and neuromodulatory compounds from the Bio-Techne brands R&D Systems, Tocris, and Novus Biologicals for neural cell culture research.

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Neural Cell Culturing Protocols

Protocol for Culturing Embryonic Rat Spinal Motor Neurons

The spinal motor neuron culture is an indispensable model system for studying neuronal development, regeneration, and the mechanisms underlying motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Motor neuron survival *in vitro* requires a specific combination of multiple growth factors and supplemental reagents. This protocol describes the use of these growth factors and supplemental reagents for successful culture of motor neurons *in vitro* together with a simple step-by-step method for isolating and culturing these neurons.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the spinal cords can be completed outside of a laminar flow cell culture hood. However, preparation of cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- Cultrex® Mouse Laminin I, Pathclear® (R&D Systems, Catalog # 3400-010-02)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- EBSS, Ca^{2+} and Mg^{2+} salts, phenol red (R&D Systems, Catalog # B31150)
- Fetal bovine serum (FBS) - Premium Select (R&D Systems, Catalog # S11550)
- L-15 (Leibovitz), L-glutamine R&D Systems, Catalog # M40150)
- Neurobasal™ medium (ThermoFisher Scientific, Catalog # 21103049), or equivalent
- NeuroXVivo™ Rat Motor Neuron Culture Kit (R&D Systems, Catalog # CDK016)
- OptiPrep™ Density Gradient Medium (Millipore Sigma, Catalog # D1556), or equivalent
- Papain (Worthington Biochemical Corp. Catalog # LK003176), or equivalent)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)

Optional Reagent:

- Cytosine β-D-arabinofuranoside (Millipore Sigma, Catalog # C1768)

Materials

- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (24-well), sterile
- Glass coverslips, 12 mm diameter round
- E14–E15 timed pregnant rat
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 100 × 20 mm
- Pipette tips

Equipment

- 37 °C, 5% (or 10%) CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Fine forceps, #5, straight (quantity 2)
  - Fine scissors, ToughCut®
  - Graefe forceps
  - Surgical scissors, small, curved
  - Vannas-Tübingen spring scissors, 2.5 mm cutting edge
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Recombinant BDNF (1000x)

1. Add 100 µL of Reconstitution Buffer 1 to the vial of Recombinant BDNF.

Recombinant CNTF (1000x)

1. Add 100 µL of Reconstitution Buffer 1 to the vial of Recombinant CNTF.

Recombinant GDNF (1000x)

1. Add 100 µL of Reconstitution Buffer 1 to the vial of Recombinant GDNF.

Recombinant Laminin α4 (1000x)

1. Add 100 µL of Reconstitution Buffer 1 to the vial of Recombinant Laminin α4.

Complete Motor Neuron Culture Media

1. Warm 10 mL of Motor Neuron Culture Media Supplement (10x) to 37 °C.
2. Add Motor Neuron Culture Media Supplement (10x) to Neurobasal™ medium to a final concentration of 1x.
3. Add Recombinant BDNF (1000x), Recombinant CNTF (1000x), Recombinant GDNF (1000x), and Recombinant Laminin α4 (1000x) to the Neurobasal™ medium plus supplement to a final concentration of 1x.

Note: Complete Motor Neuron Culture Media is best used within 1 week. Scale up or down to make the desired amount of Complete Motor Neuron Culture Media for the experiment.

Procedure

Coating of Cell Culture Plates

Note: Rat motor neurons can be cultured on Laminin I, poly-D-lysine-coated glass coverslips or directly onto tissue culture plates coated with Laminin I and poly-D-lysine. If using pre-coated coverslips or plates, skip this section.

Note: Preparation of the cell culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.

2. Add 1 mL of the 50 µg/mL Cultrex® Poly-D-Lysine solution to each well of the cell culture plates. Tilt the plates gently to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. After the third wash, aspirate the wells to completely remove all liquids.
5. Wrap plates with Parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Start the remaining steps the day before collection of the rat spinal cord tissue.

6. Dilute the Cultrex® Mouse Laminin I solution with sterile PBS to a final concentration of 10 µg/mL.
7. Cover the wells containing poly-D-lysine-coated coverslips with 10 µg/mL Cultrex® Mouse Laminin I (e.g. 0.5 mL/well for 24-well plate). Tilt the plates to ensure even coating of the well surface.
8. Incubate the plates overnight at 2–8 °C.
9. Aspirate the Laminin I solution from the wells prior to adding the cells. Wash the wells two times with sterile dH_2O. Aspirate the wells to remove all liquids.

Note: Alternatively, spinal motor neurons can be cultured on pre-coated glass coverslips, such as mouse Laminin I and poly-D-lysine coated glass coverslips, 12 mm diameter. Place one pre-coated coverslip into each well of a 24-well cell culture plate.

Dissection of Embryonic Rat Spinal Cords

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount of L-15 medium and Complete Motor Neuron Culture Media in a 37 °C water bath.
2. Place sterile PBS on ice.
3. Asphyxiate pregnant rat with CO_2. Recover embryos via cesarean section using the fine scissors and Graefe forceps. Place the embryos in a 100 × 20 mm petri dish containing cold PBS. Keep the dish on ice.
4. Remove the embryos from their individual placenta sacs and wash with cold PBS.
5. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold PBS. Carefully decapitate each embryo at the head/neck junction using a curved, small surgical scissors. Discard the heads.
6. Place the body of the embryo with the dorsal side up. Position the embryo so that the tail is pointed towards you.
7. With the fine scissors, cut off the tail. Under a dissecting microscope, carefully remove skin and tissue with a #5 fine forceps, moving in a ventral direction, until the dorsal surface of the spinal cord is visible.
8. Moving caudal to rostral, cut along the dorsal midline of the spinal cord with the Vannas-Tübingen spring scissors to "open" the spinal cord, separating the left and right sides.
9. Using two #5 fine forceps, one to hold the body on one side and the other to remove surrounding tissue from the other side of the spinal cord, isolate the spinal cord and expose the dorsal root ganglia (DRG). Remove the DRG and meninges by rubbing the forceps between the spinal cord and the DRG and meninges. Repeat this process on the other side to free the spinal cord.
10. Remove the spinal cord from the body by grabbing onto one end of the spinal cord and lifting it up and away from the body. Use the Vannas-Tübingen spring scissors to cut along the dorsal-ventral midline to trim off the dorsal part of the spinal cord.
11. While holding the remaining ventral spinal cord, cut along the ventral midline to bisect it.
12. Transfer the isolated ventral spinal cord to a clean 100 × 20 mm dish containing cold L-15 medium. Mince the spinal cord into small pieces with the Vannas-Tübingen spring scissors.

Dissociation and Culture of Embryonic Rat Spinal Motor Neurons

Note: From this point forward, any opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

1. Transfer the minced spinal cord tissue and L-15 medium to a 15 mL conical tube. Centrifuge at 200 × g for 3 minutes at room temperature. Decant the medium from the pelleted tissue pieces.
2. In a 15 mL conical tube, mix 20 U/mL Papain and 100 U/mL DNase I in 5 mL of EBSS. Warm the solution in a 37 °C, 5% CO_2 humidified incubator for 15 minutes.
3. Transfer the warmed enzyme solution to the tube containing the pelleted tissue pieces. Incubate for 15–20 minutes in a 37 °C, 5% CO_2 humidified incubator.
4. Add 3 mL of FBS to the 15 mL conical tube to inactivate the enzyme solution. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution from the pelleted tissue pieces.
5. Add 6 mL of L-15 medium to the 15 mL conical tube. Gently triturate the tissue pieces with the fire-polished Pasteur pipette until the solution is homogenous.

Note: Avoid generating bubbles while triturating.

6. Prepare 20 mL of 9% OptiPrep™ solution in L-15 medium in a 50 mL conical tube. Transfer 3 mL of the solution to each of six 15 mL conical tubes.
7. Divide the homogenized solution evenly among the six tubes containing the OptiPrep™ solution. Centrifuge the tubes at 900 × g for 15 minutes at room temperature.

Note: Brakes should be off during this centrifugation step.

8. Carefully collect the top 2 mL of solution from each tube and pool into a 50 mL conical tube. Fill the 50 mL conical tube with L-15 medium. Centrifuge at 300 × g for 10 minutes at room temperature. Remove and save the solution from the pelleted cells.

9. Resuspend the cells in 250–500 µL of Complete Motor Neuron Culture Media. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells and resuspend at a density of 0.5–1.0 × 10^5 cells/mL.

Note: If cell count is too low, centrifuge the saved solution from step 8 at 300 × g for 10 minutes at room temperature. Resuspend the pelleted cells with the cell solution from step 9. Repeat cell count.

10. Cover the previously prepared coverslips with 80 µL of Complete Motor Neuron Culture Media. Add 20 µL of the cell suspension to each coverslip.
11. Incubate the cell culture plates in a 37 °C, 5% CO_2 humidified incubator for at least 2 hours to allow the cells to adhere to the coverslips.
12. Carefully add 900 µL of Complete Motor Neuron Culture Media to each well of the cell culture plate.
13. Keep cultured spinal motor neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in Spinal Motor Neuron Cultures

Note: Culture motor neurons at 37 °C, 5% CO_2 humidified incubator for 14 days. Exchange the media every 3–4 days.

1. Warm an appropriate amount of Complete Motor Neuron Culture Media in a 37 °C, 5% CO_2 humidified incubator.
2. Gently remove half the volume of media (i.e. 500 µL) from each well of the cell culture plates. Gently add 500 µL of new, warmed Complete Motor Neuron Culture Media to each well of the cell culture plates.

Note: Do not remove all the media from the wells of the cell culture plate as this will stress the spinal motor neurons.

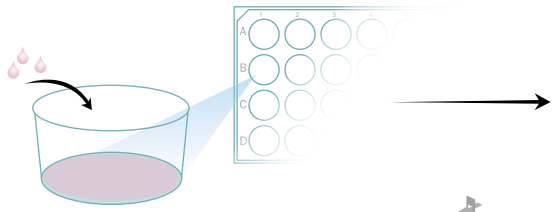
3. Exchange the media every 3–4 days.

Note: To suppress glial cell growth, add 100 nM of cytosine β-D-arabinofuranoside to the spinal motor neuron cultures during all media exchanges.

Protocol for Culturing Embryonic Rat Spinal Motor Neurons

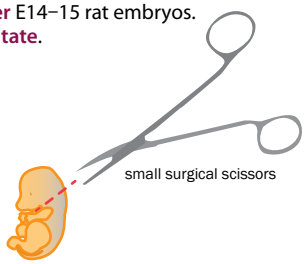
Day 1

Prepare cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.



Day 2

Recover E14–15 rat embryos. **Decapitate**.



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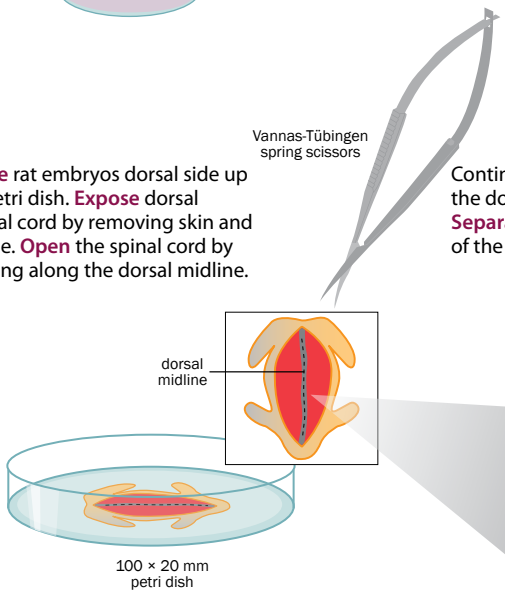
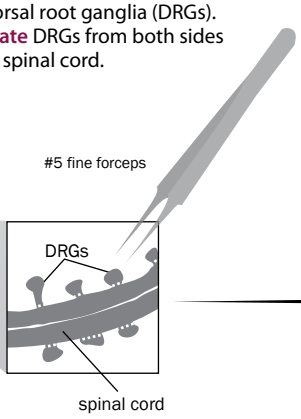
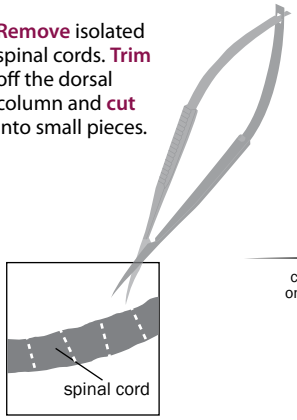
Place rat embryos dorsal side up in petri dish. **Expose** dorsal spinal cord by removing skin and tissue. **Open** the spinal cord by cutting along the dorsal midline.



Continue to **remove** tissue to expose the dorsal root ganglia (DRGs). **Separate** DRGs from both sides of the spinal cord.



Remove isolated spinal cords. **Trim** off the dorsal column and **cut** into small pieces.



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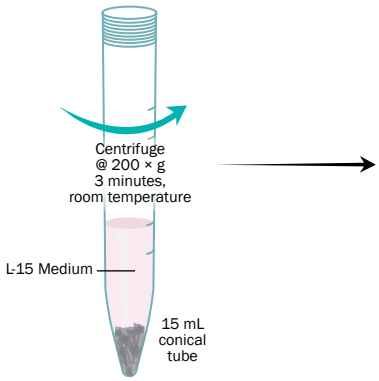
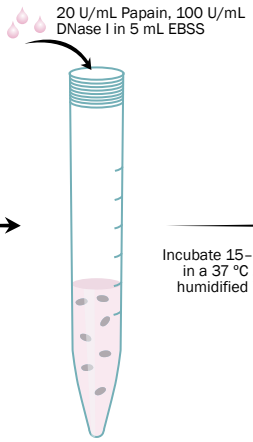
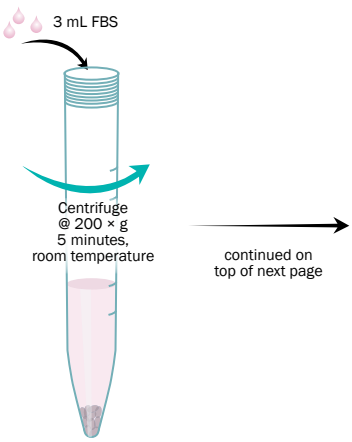
Transfer dissected spinal cord tissue. **Centrifuge**. **Decant** supernatant.



Digest the spinal cord tissue.



Stop the tissue digestion. **Centrifuge**. **Decant** supernatant.



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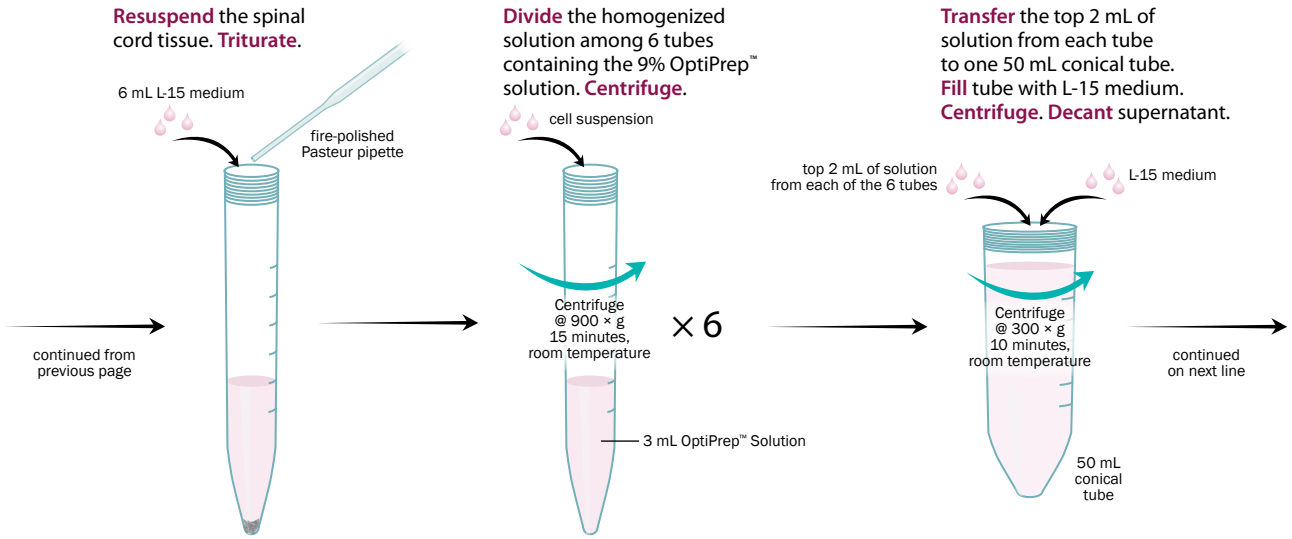
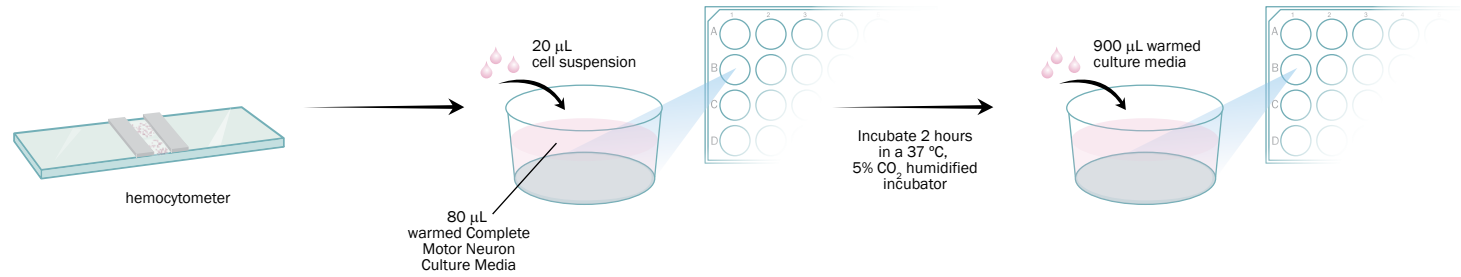
Protocol for Culturing Embryonic Rat Spinal Motor Neurons, continued





Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons

Dorsal root ganglion (DRGs) neurons are somatosensory neurons that reside in ganglions on the dorsal root of the spinal cord. Chick DRG culture is an indispensable model system for studying neurite outgrowth, regeneration, and degeneration, as well as the molecular mechanisms of nociception and myelination in the central and peripheral nervous systems. This protocol provides step-by-step instructions for dissecting and culturing a semi-pure DRG culture.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this cell culture protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of DRGs can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- 70% ethanol
- Cultrex® Mouse Laminin I, Pathclear® (R&D Systems, Catalog # 3400-010-02) or Bovine Fibronectin Protein (R&D Systems, Catalog # 1030-FN)
- DMEM, high glucose, no glutamine, no sodium, pyruvate (R&D Systems, Catalog # M22850)
- Fetal bovine serum (FBS) - Premium Select R&D Systems, Catalog # S11550)
- L-glutamine-penicillin-streptomycin solution (100x), or equivalent
- Ham's F-12K (Kaighn's) medium
- HEPES Buffer Solution (1 M, R&D Systems, Catalog # B35110)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Recombinant Human β-NGF (R&D Systems, Catalog # 256-GF)
- Sodium pyruvate (100 mM)
- Trypan blue (0.4%)
- Trypsin 2.5% (10x, R&D Systems, Catalog # B81710)

Materials

- 15 mL conical centrifuge tubes, sterile
- Alcohol pads
- Cell culture plates (96-well), sterile
- E10–E13 embryonated Leghorn chicken eggs
- Ice
- Paper towel
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Dissecting forceps, straight (e.g. Gillies)
  - Fine forceps, #5, straight
  - Fine forceps, #5/45, angled 45°
  - Forceps, #7, curved
  - Scissors, extra narrow, straight, sharp tip
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Dissection Media

1. 1x L-glutamine-penicillin-streptomycin solution, 20 mM HEPES buffer solution in Ham's F-12K medium

Plating Media

1. 1x L-glutamine-penicillin-streptomycin solution, 1 mM sodium pyruvate, 10 mM HEPES buffer solution, 10% FBS in a Ham's F-12K medium/DMEM (1:1) solution

Culture Media

1. 1x L-glutamine-penicillin-streptomycin solution, 1 mM sodium pyruvate, 10 mM HEPES buffer solution, 1x N-2 Plus Media Supplement, 1 ng/mL Recombinant Human β-NGF in a Ham's F-12K medium/DMEM (1:1) solution

Procedure

Coating of Cell Culture Plate

Note: Preparation of the cell culture plates should be done in a laminar flow cell culture hood.

1. Prepare a 15 µg/mL solution of either Mouse Laminin I or Bovine Fibronectin Protein in sterile PBS.
2. Add 50 µL of the solution to each well of a cell culture plate. Incubate the plate overnight at 2–8 °C.
3. Wash the wells twice with 100 µL/well of sterile PBS. Add 50 µL of culture media to each well of the cell culture plate. Incubate the plate in a 37 °C, 5% CO_2 humidified incubator for 30 minutes. Plate is ready for DRG neurons to be seeded.

Dissection of Embryonic Chick DRGs

Note: Soak dissection tools in 70% ethanol for 20–30 minutes to sterilize. Place the tools on a paper towel and let air dry before use.

1. Place sterile PBS and dissection media on ice.
2. Wipe an embryonated egg with an alcohol pad.
3. Holding the egg with the air sack on top, crack the eggshell. Pull the chicken embryo out using the dissecting forceps and place in a 60 × 15 mm petri dish. Decapitate the embryo using the extra narrow scissors and discard the head.
4. With the embryo on its back, clear the spinal cord of all the visceral tissues and organs using the #7 curved forceps. Rinse the embryo cavity with sterile PBS and then dissection media.
5. Under a dissecting microscope, dissect out the DRGs all along the spinal cord using the #5 fine forceps and place in a 60 × 15 mm petri dish containing fresh, cold dissection media. Use the #5 fine forceps and the #5/45 fine forceps to remove the extraneous tissues from the isolated DRGs.

Note: Do not puncture the outside membrane of the DRGs.

6. Transfer the clean DRGs to a separate 15 mL conical tube containing cold dissection media. Keep DRGs on ice until dissection is complete.
7. Repeat steps 3–7 for 4 to 7 chicken embryos, which should provide enough DRG neurons for one 96-well cell culture plate.
8. After all DRGs have been isolated, centrifuge the 15 mL conical tube at 193 × g for 3–5 minutes at room temperature.

Note: From this point forward, the opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

9. Discard the supernatant.

Dissociation and Culture of Embryonic Chick DRGs

1. Warm an appropriate amount of plating and culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Resuspend the DRGs in 4.8 mL of sterile PBS. Add 200 µL of 2.5% Trypsin with no EDTA. Mix the contents of the 15 mL conical tube by gentle agitation. Incubate the tube in a 37 °C water bath for 4–10 minutes, gently agitating the tube several times during the incubation.

Note: The length of this trypsinization step will vary depending on the number of DRGs that were isolated. The incubation period is over once the DRGs clump together, at which point, the 15 mL conical tube can be removed from the 37 °C water bath.

3. Add 7 mL of plating media to the 15 mL conical tube. Centrifuge at 200 × g for 3–5 minutes at room temperature. Discard the supernatant.
4. Resuspend DRGs in 4–5 mL of plating media. Dissociate the DRG tissue into a single cell suspension by trituration with the fire-polished Pasteur pipette.
5. Add 8–9 mL of plating media. Transfer the cell suspension to a 100 × 20 mm petri dish. Incubate the dish for 3–4 hours in a 37 °C, 5% CO_2 humidified incubator.

Note: During this incubation, non-neuronal cells will attach to the bottom of the petri dish while the DRG neurons will remain in suspension.

6. Collect and transfer the plating media (hence, the non-attached DRG neurons) to a 15 mL conical tube. Centrifuge at 200 × g for 5–7 minutes at room temperature. Discard the supernatant.
7. Resuspend the neurons in 2–5 mL of culture media. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
8. Reconstitute the cells with culture media to a concentration of 15–20 × 10^4 cells/mL. Add 100 µL of the cell suspension to each well of a prepared cell culture plate so there are 15,000–20,000 cells/well.
9. Keep the cultured DRG neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in DRG Neuron Cultures

1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Gently remove half the volume of media (i.e. 50 µL) from each well of the cell culture plate. Gently add 50 µL of new, warmed culture media to each well of the cell culture plate.

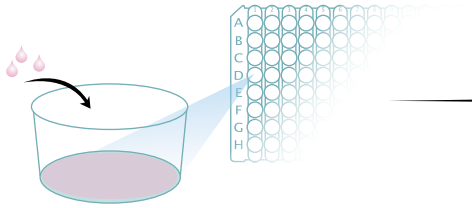
Note: Do not remove all the media from the wells of the cell culture plate as this will stress the DRG neurons.

3. Exchange the culture media every 3–4 days.

Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons

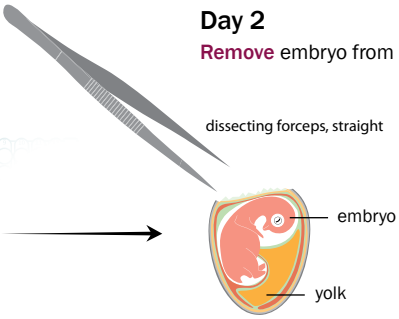
Day 1

Prepare cell culture plates by coating with Mouse Laminin I or Bovine Fibronectin Protein.

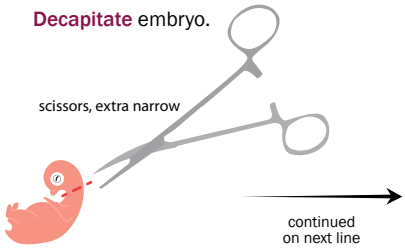


Day 2

Remove embryo from egg.



Decapitate embryo.



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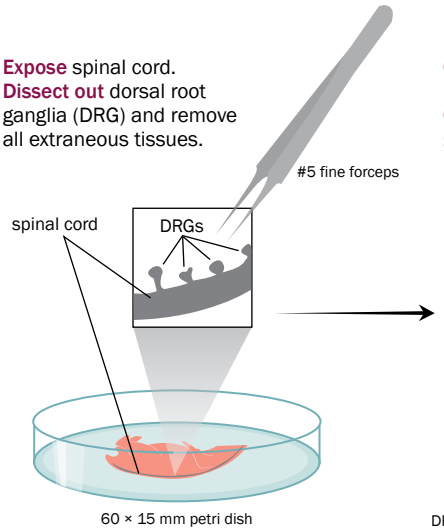
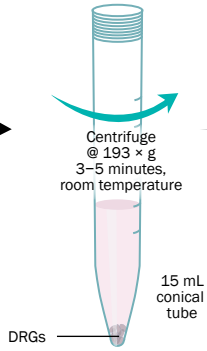
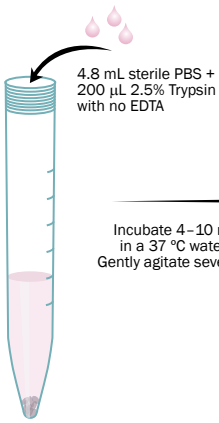
Expose spinal cord.
Dissect out dorsal root ganglia (DRG) and remove all extraneous tissues.



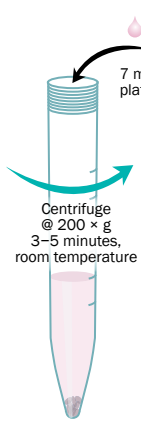
Collect cleaned DRGs in cold dissection media.
Centrifuge. **Decant** supernatant.



Resuspend DRGs.
Mix by gentle agitation.



Add warmed plating media.
Centrifuge. **Decant** supernatant.



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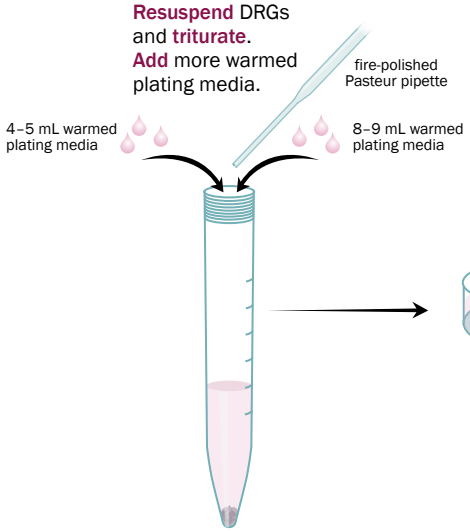
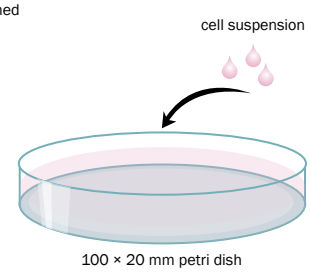
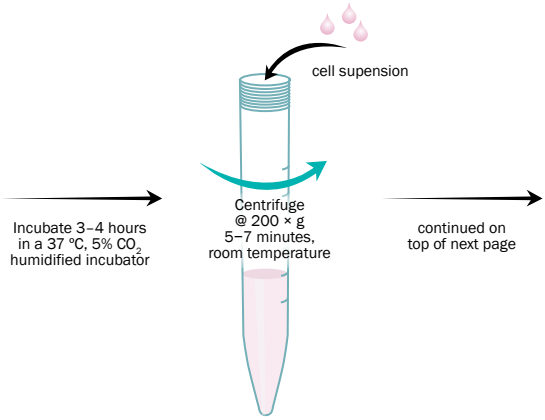
Resuspend DRGs and **triturate**.
Add more warmed plating media.



Transfer cell suspension.



Transfer DRG neurons.
Centrifuge. **Decant** supernatant.



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Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons, continued

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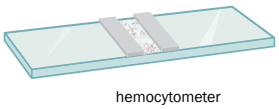
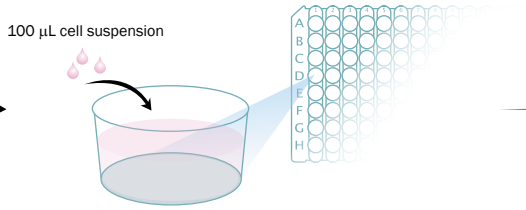
Resuspend DRGs neurons in 2–5 mL warmed culture media. **Count** cells.



hemocytometer

Reconstitute DRG neurons with warmed culture media. **Seed** neurons onto coated cell culture plates.



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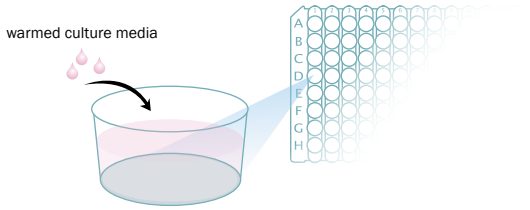
Culture DRG neurons for desired amount of time. **Exchange** media every 3–4 days.



Protocol for Culturing Rat Cortical Neurons

Cortical neural cell cultures are an important model system for studying neuronal development and function, neurotoxicity screening, drug discovery, and mechanisms of neurological diseases. Proper development and survival of neurons, requires specific growth factors, signaling molecules, peptides, and vitamins.[1,2] This protocol provides step-by-step instructions for dissecting and culturing cortical neurons from both embryonic (E17–E18) and postnatal (P1–P2) rat pups.

Please read the protocol in its entirety before starting.

Note: In order to yield a healthy neuron population, brain tissue from P1–P2 rat pups needs to be enzymatically digested before trituration. Extra reagents and steps are required.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the cortical tissue can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- Antibiotic-Antimycotic (100x, R&D Systems, Catalog # B22110)
- Cultrex® Mouse Laminin I, Pathclear® (R&D Systems, Catalog # 3400-010-02)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- DMEM, high glucose, no glutamine, no sodium pyruvate* (R&D Systems, Catalog # M22850)
- L-Glutamine - 200 mM (100x, R&D Systems, Catalog # B90010)
- N21-MAX Media Supplement (50x, R&D Systems, Catalog # AR008)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)

Additional Reagents for P1–P2 Rat Pups:

- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- EBSS, Ca^{2+} and Mg^{2+} salts, phenol red (R&D Systems, Catalog # B31150)
- Ovomucoid protease inhibitor with BSA (Worthington Biochemical Corp., Catalog # LK003182), or equivalent
- Papain (Worthington Biochemical Corp., Catalog # LK003176), or equivalent

Optional Reagents:

- Recombinant Human IGF-I/IGF-1 Protein (R&D Systems, Catalog # 291-G1)
- Recombinant Human BDNF Protein (R&D Systems, Catalog # 11166-BD)

Materials

- 15 mL conical centrifuge tube
- Cell culture plates
- E17–E18 timed pregnant rat or P1–P2 rat pups
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips

Note: Locate your desired seeding densities for your cortical cell culture in Table 1 (pg. 34) to determine the size of the cell culture plate that should be used.

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Dissecting forceps, curved (e.g. Gillies)
  - Fine forceps, #5, straight
  - Forceps, #7, curved
  - Surgical scissors, small
  - Surgical scissors, large
  - Vannas-Tübingen spring scissors, straight
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Culture Media

1. 1x N21-MAX Media Supplement, 1x Antibiotic-Antimycotic, 0.5 mM L-Glutamine in DMEM medium

Note: Recombinant Human BDNF Protein and Recombinant Human IGF-I/IGF-1 Protein can be added to enhance the cortical cell culture.

Procedure

Coating and Preparation of Cell Culture Plates

Note: Preparation of the culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.
2. Cover the wells of the culture plates with 50 µg/mL Cultrex® Poly-D-Lysine solution (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. Aspirate the wells to remove all liquids.
5. Wrap plates with Parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Start these remaining steps the day before collection of the rat cortical tissue.

6. Dilute the Cultrex® Mouse Laminin I solution with sterile PBS to a final concentration of 10 µg/mL.
7. Cover the wells of the poly-D-lysine-coated plates with 10 µg/mL Cultrex® Mouse Laminin I (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
8. Incubate the plates overnight at 2–8 °C.

*Neurobasal™ medium can be used in place of DMEM.

9. Aspirate the mouse Laminin I solution from the wells prior to adding the cells. Wash the wells two times with sterile dH_2O. Aspirate the wells to remove all liquids.

Note: Alternatively, cortical neurons can be cultured on poly-L-lysine coated, open μ-Slides (chambered #1.5 polymer coverslips; ibidi, Catalog # 80824).

Dissection of Rat Cortical Tissue

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount of DMEM medium and culture media in a 37 °C water bath. Place sterile PBS on ice.

Note: If using P1–P2 rat pups, skip to step 4.

2. Asphyxiate the pregnant rat with CO_2. Recover embryos via cesarean section using the large surgical scissors and curved dissecting forceps. Place the embryos in a 100 × 20 mm petri dish containing cold PBS. Keep the dish on ice.
3. Remove the embryos from their individual placenta sacs and wash with cold PBS.
4. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold PBS. Decapitate each embryo at the head/neck junction using the small surgical scissors. Decapitate P1–P2 rat pups with the small surgical scissors.
5. Place the heads in a new 100 × 20 mm petri dish containing cold PBS.
6. Stabilize the dissociated head using the #7 curved forceps and #5 fine forceps. Moving caudal to rostral, cut through the skull with the small surgical scissors.

Note: Keep cuts shallow to avoid damaging the brain tissue.

7. Peel back the two halves of the separated skull.
8. Remove the whole brain from the head cavity using the #7 curved forceps and place in a 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 4–8 for the remaining heads.
9. Put a brain in a new 60 × 15 mm petri dish containing cold PBS. Under a dissecting microscope, cut the brain with the Vannas-Tübingen spring scissors, following the median longitudinal fissure, to separate the hemispheres. Cut off and discard any brainstem and cerebellar tissue.
10. With the #5 fine forceps, peel off the meninges that cover each hemisphere. Open up the brain to reveal the mid-sagittal side.
11. Locate the hippocampus, which is the darker, c-shaped region, and remove it using the Vannas-Tübingen spring scissors. Place the remaining cortical tissue in a new 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 9–11 for the remaining brains.
12. Using the Vannas-Tübingen spring scissors, cut the isolated cortical tissue into smaller pieces (~2 mm^2).

Dissociation and Culture of Rat Cortical Neurons

Note: From this point forward, the opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

Note: If using embryonic cortical tissue, start at step 1. If using P1–P2 tissue, start at step 3.

For embryonic cortical tissue:

1. Transfer the tissue pieces to a 15 mL conical tube. Add 5 mL of DMEM medium. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
2. Skip to step 6.

For postnatal cortical tissue:

3. In a 15 mL conical tube, mix 20 U/mL Papain and 100 U/mL DNase I in 5 mL of EBSS. Warm the solution in a 37 °C, 5% CO_2 humidified incubator for 10 minutes.
4. Transfer the tissue to the 15 mL conical tube containing the warmed enzyme solution. Incubate for 20–30 minutes in a 37 °C, 5% CO_2 humidified incubator.
5. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
6. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution.
7. Resuspend the cells in...

   For embryonic cortical tissue:
   10 mL of DMEM medium.

   For postnatal cortical tissue:
   5 mL of EBSS containing 1 μg/mL of Ovomucoid protease inhibitor with BSA.

8. Centrifuge at 200 × g for 4–6 minutes at room temperature. Decant the solution.
9. Wash the cells twice with 10 mL of DMEM medium. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the media.
10. Resuspend the cells in warmed culture media (~ 10 mL). Mix 10 μL of the cell suspension with 10 μL of 0.4% Trypan blue. Count the live cells.
11. Dilute the cell suspension to the desired seeding density (see Table 1, pg. 34) with warmed culture media. Plate the neurons on the prepared culture plates.
12. Keep cultured neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in Cortical Neuron Cultures

Note: Healthy cultures can be maintained for up to 4 weeks.

1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Remove half the volume of media from each well of the culture plate (e.g., remove 50 μL from each well of a 96-well plate). Gently add an equal amount of new, warmed, culture media to each well.

Note: Do not remove all the media from the wells of the plate as this will stress the neurons.

3. Exchange the culture media every 3–4 days.

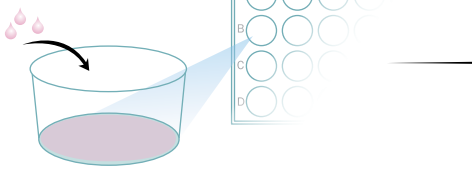
References

1. Catapano, L.A. *et al.* (2001) J. Neurosci. **21**:8863.
2. Martin, D.L. (1992) Glia **5**:81.

Protocol for Culturing Rat Cortical Neurons

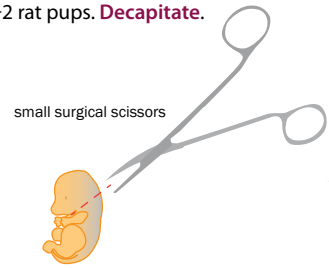
Day 1

Prepare cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.



Day 2

Isolate E17–18 rat embryos or P1–2 rat pups. **Decapitate**.



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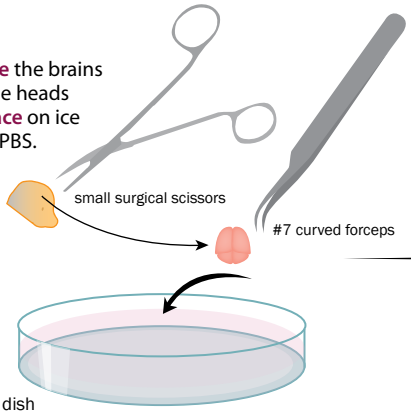
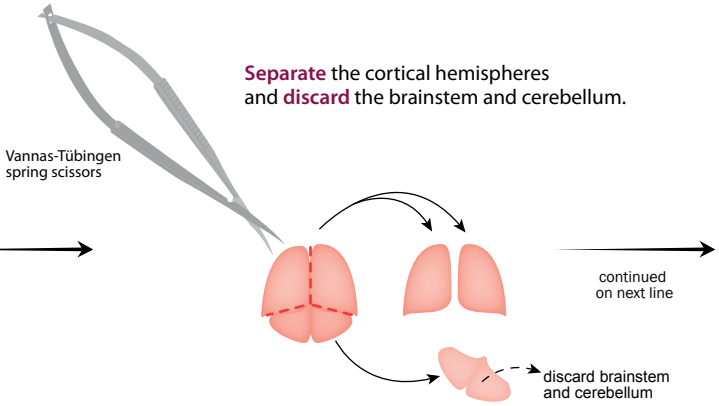
Remove the brains from the heads and **place** on ice in cold PBS.



Separate the cortical hemispheres and **discard** the brainstem and cerebellum.



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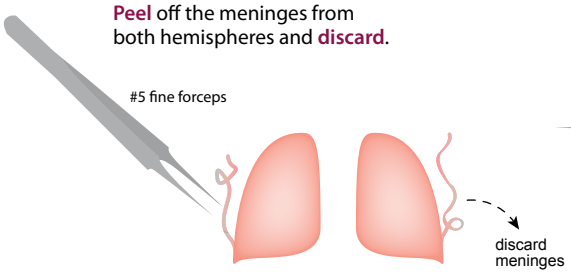
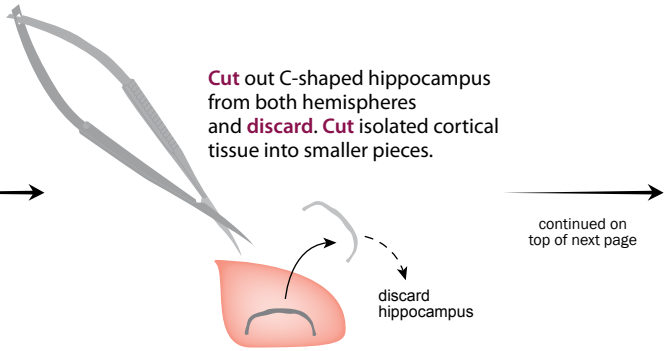
Peel off the meninges from both hemispheres and **discard**.



Cut out C-shaped hippocampus from both hemispheres and **discard**. **Cut** isolated cortical tissue into smaller pieces.



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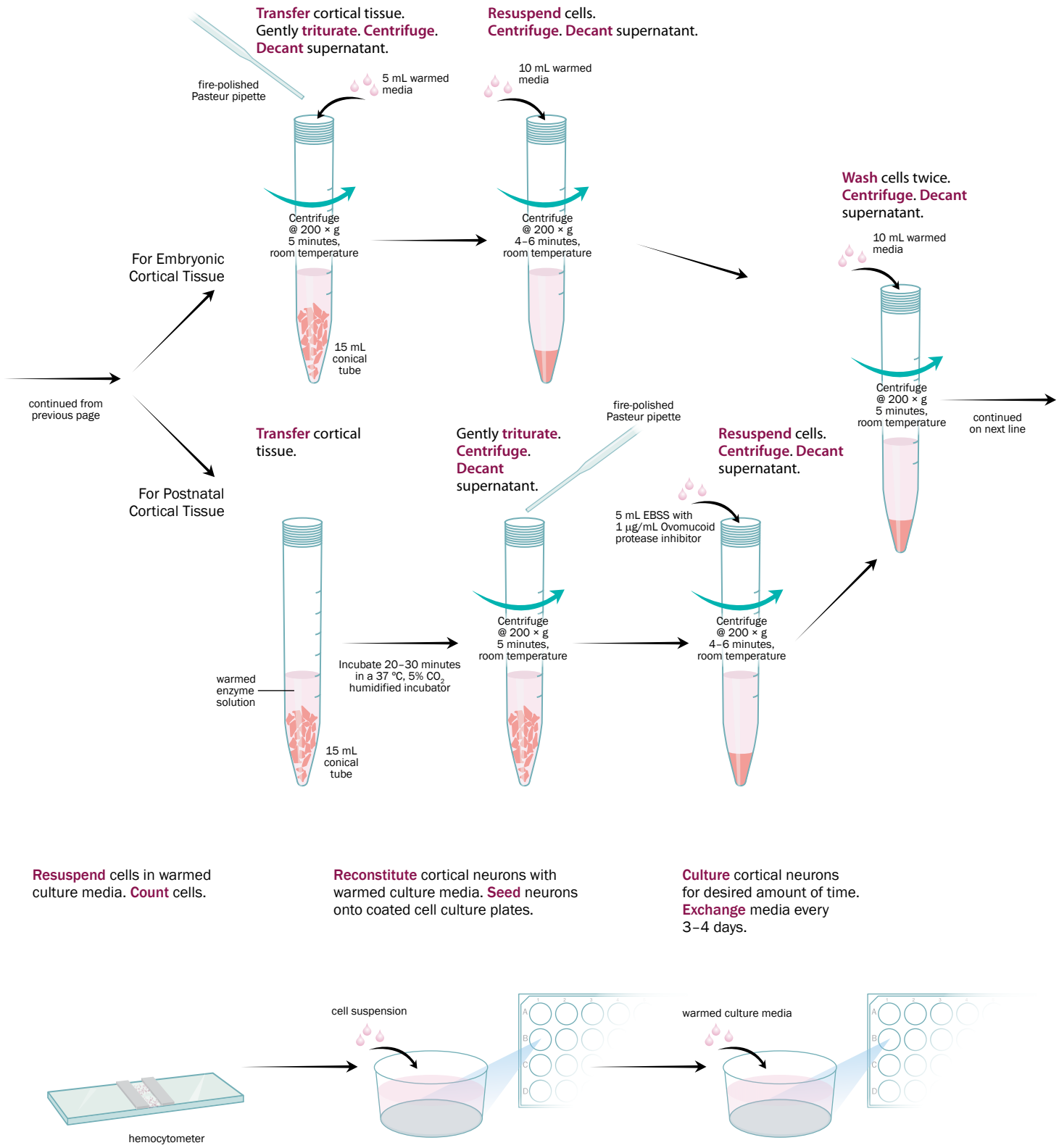
Protocol for Culturing Rat Cortical Neurons, continued



Resuspend cells in warmed culture media. **Count** cells.

Reconstitute cortical neurons with warmed culture media. **Seed** neurons onto coated cell culture plates.

Culture cortical neurons for desired amount of time. **Exchange** media every 3–4 days.

Protocol for Culturing Rat Hippocampal Neurons

Hippocampal neural cell cultures are a commonly used model system for not only investigating the physiological properties of learning and memory, but the cellular mechanisms of neurobiology in general. Hippocampal cell cultures contain relatively few interneurons, and these interneurons are morphologically distinguishable from pyramidal neurons, the major cell type in the hippocampus[1]. Another benefit of hippocampal cell cultures is that they form fully developed dendrites that are covered with spines and make substantial synaptic connections. This protocol provides step-by-step instructions for dissecting and culturing hippocampal neurons from both embryonic (E17–E18) and postnatal (P1–P2) rat pups.

Please read the protocol in its entirety before starting.

Note: In order to yield a healthy neuron population, brain tissue from P1–P2 rat pups needs to be enzymatically digested before trituration. Extra reagents and steps are required.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the hippocampi can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- Antibiotic-Antimycotic (100x, R&D Systems, Catalog # B22110)
- Cultrex® Mouse Laminin I , Pathclear® (R&D Systems, Catalog # 3400-010-02)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- DMEM, high glucose, no glutamine, no sodium pyruvate* (R&D Systems, Catalog # M22850)
- L-Glutamine - 200 mM (100x, R&D Systems, Catalog # B90010)
- N21-MAX Media Supplement (50x, R&D Systems, Catalog # AR008)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)

Additional Reagents for P1–P2 Rat Pups:

- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- EBSS, Ca^{2+} and Mg^{2+} salts, phenol red (R&D Systems, Catalog # B31150)
- Ovomucoid protease inhibitor with BSA (Worthington Biochemical Corp., Catalog # LK003182), or equivalent
- Papain (Worthington Biochemical Corp., Catalog # LK003176), or equivalent

Optional Reagents:

- Recombinant Human IGF-I/IGF-1 Protein (R&D Systems, Catalog # 291-G1)
- Recombinant Human BDNF Protein (R&D Systems, Catalog # 11166-BD)

Materials

- 15 mL conical centrifuge tube
- Cell culture plates
- E17–E18 timed pregnant rat or P1–P2 rat pups
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips

Note: Locate your desired seeding densities for your hippocampal cell culture in Table 1 (pg. 34) to determine the size of the cell culture plate that should be used.

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Dissecting forceps, curved (e.g. Gillies)
  - Fine forceps, #5, straight
  - Forceps, #7, curved
  - Surgical scissors, small
  - Surgical scissors, large
  - Vannas-Tübingen spring scissors, straight
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

*Neurobasal™ medium can be used in place of DMEM.

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Culture Media

1. 1x N21-MAX Media Supplement, 1x Antibiotic-Antimycotic, 0.5 mM L-Glutamine in DMEM medium

Note: Recombinant Human BDNF Protein and Recombinant Human IGF-I/IGF-1 Protein can be added to enhance the hippocampal cell culture.

Procedure

Coating and Preparation of Cell Culture Plates

Note: Preparation of the culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.
2. Cover the wells of the culture plates with 50 µg/mL Cultrex® Poly-D-Lysine solution (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. Aspirate the wells to remove all liquids.
5. Wrap plates with Parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Start these remaining steps the day before collection of the rat hippocampi.

6. Dilute the Cultrex® Mouse Laminin I solution with sterile PBS to a final concentration of 10 µg/mL.
7. Cover the wells of the poly-D-lysine-coated plates with 10 µg/mL Cultrex® Mouse Laminin I (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
8. Incubate the plates overnight at 2–8 °C.

9. Aspirate the mouse Laminin I solution from the wells prior to adding the cells. Wash the wells two times with sterile dH_2O. Aspirate the wells to remove all liquids.

Note: Alternatively, hippocampal neurons can be cultured on poly-L-lysine coated, open μ-Slides (chambered #1.5 polymer coverslips; ibidi, Catalog # 80824).

Dissection of Rat Hippocampi

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount of DMEM medium and culture media in a 37 °C water bath. Place sterile PBS on ice.

Note: If using P1–P2 rat pups, skip to step 4.

2. Asphyxiate the pregnant rat with CO_2. Recover embryos via cesarean section using the large surgical scissors and curved dissecting forceps. Place the embryos in a 100 × 20 mm petri dish containing cold PBS. Keep the dish on ice.
3. Remove the embryos from their individual placenta sacs and wash with cold PBS.
4. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold PBS. Decapitate each embryo at the head/neck junction using the small surgical scissors. Decapitate P1–P2 rat pups with the small surgical scissors.
5. Place the heads in a new 100 × 20 mm petri dish containing cold PBS.
6. Stabilize the dissociated head using the #7 curved forceps and #5 fine forceps. Moving caudal to rostral, cut through the skull with the small surgical scissors.

Note: Keep cuts shallow to avoid damaging the brain tissue.

7. Peel back the two halves of the separated skull.
8. Remove the whole brain from the head cavity using the #7 curved forceps and place in a 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 4–8 for the remaining heads.
9. Put a brain in a new 60 × 15 mm petri dish containing cold PBS. Under a dissecting microscope, cut the brain with the Vannas-Tübingen spring scissors, following the median longitudinal fissure, to separate the hemispheres. Cut off and discard any brainstem and cerebellar tissue.
10. With the #5 fine forceps, peel off the meninges that cover each hemisphere. Open up the brain to reveal the mid-sagittal side.
11. Locate the hippocampus, which is the darker, c-shaped region, and remove it using the Vannas-Tübingen spring scissors. Place the hippocampal tissue in a new 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 9–11 for the remaining brains.
12. Using the Vannas-Tübingen spring scissors, cut the isolated hippocampi into smaller pieces (~2 mm^2).

Dissociation and Culture of Rat Hippocampal Neurons

Note: From this point forward, the opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

Note: If using embryonic hippocampal tissue, start at step 1. If using P1–P2 tissue, start at step 3.

For embryonic hippocampi:

1. Transfer the tissue pieces to a 15 mL conical tube. Add 5 mL of DMEM medium. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
2. Skip to step 6.

For postnatal hippocampi:

3. In a 15 mL conical tube, mix 20 U/mL Papain and 100 U/mL DNase I in 5 mL of EBSS. Warm the solution in a 37 °C, 5% CO_2 humidified incubator for 10 minutes.
4. Transfer the tissue to the 15 mL conical tube containing the warmed enzyme solution. Incubate for 20–30 minutes in a 37 °C, 5% CO_2 humidified incubator.
5. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
6. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution.
7. Resuspend the cells in…

   For embryonic hippocampi:
   10 mL of DMEM medium.

   For postnatal hippocampi:
   5 mL of EBSS containing 1 μg/mL of Ovomucoid protease inhibitor with BSA.

8. Centrifuge at 200 × g for 4–6 minutes at room temperature. Decant the solution.
9. Wash the cells twice with 10 mL of DMEM medium. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the media.
10. Resuspend the cells in warmed culture media (~ 10 mL). Mix 10 μL of the cell suspension with 10 μL of 0.4% Trypan blue. Count the live cells.
11. Dilute the cell suspension to the desired seeding density (see Table 1, pg. 34) with warmed culture media. Plate the neurons on the prepared culture plates.
12. Keep cultured neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in Hippocampal Neuron Cultures

Note: Healthy cultures can be maintained for up to 4 weeks.

1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Remove half the volume of media from each well of the culture plate (e.g., remove 50 μL from each well of a 96-well plate). Gently add an equal amount of new, warmed, culture media to each well.

Note: Do not remove all the media from the wells of the plate as this will stress the neurons.

3. Exchange the culture media every 3–4 days.

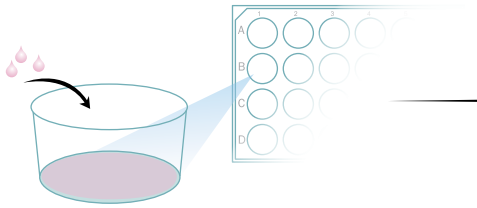
References

1. Kaech, S. and G. Banker (2006) Nat. Protoc. **1**:2406.

Protocol for Culturing Rat Hippocampal Neurons

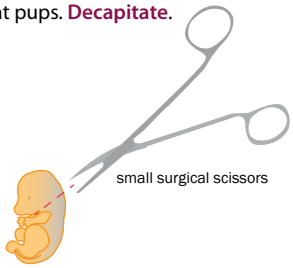
Day 1

Prepare cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.



Day 2

Isolate E17–18 rat embryos or P1–2 rat pups. **Decapitate**.



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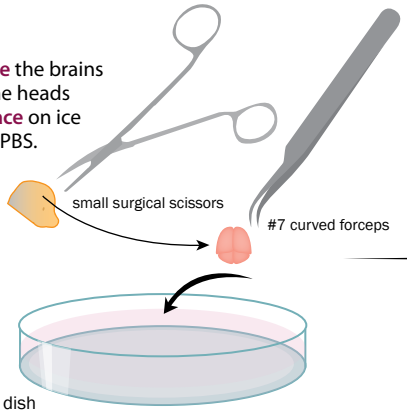
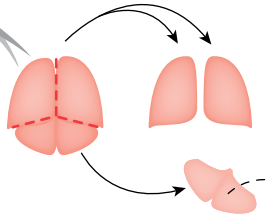
Remove the brains from the heads and **place** on ice in cold PBS.



60 × 15 mm petri dish

Vannas-Tübingen spring scissors

Separate the cortical hemispheres and **discard** the brainstem and cerebellum.



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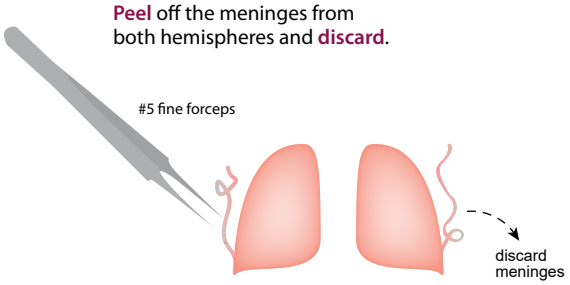
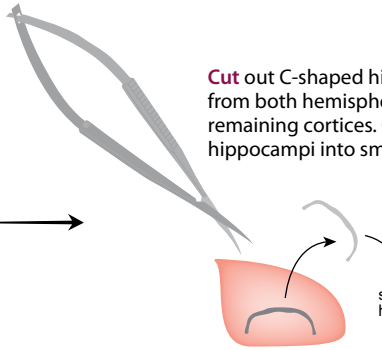
Peel off the meninges from both hemispheres and **discard**.



Cut out C-shaped hippocampus from both hemispheres. **Discard** remaining cortices. **Cut** isolated hippocampi into smaller pieces.



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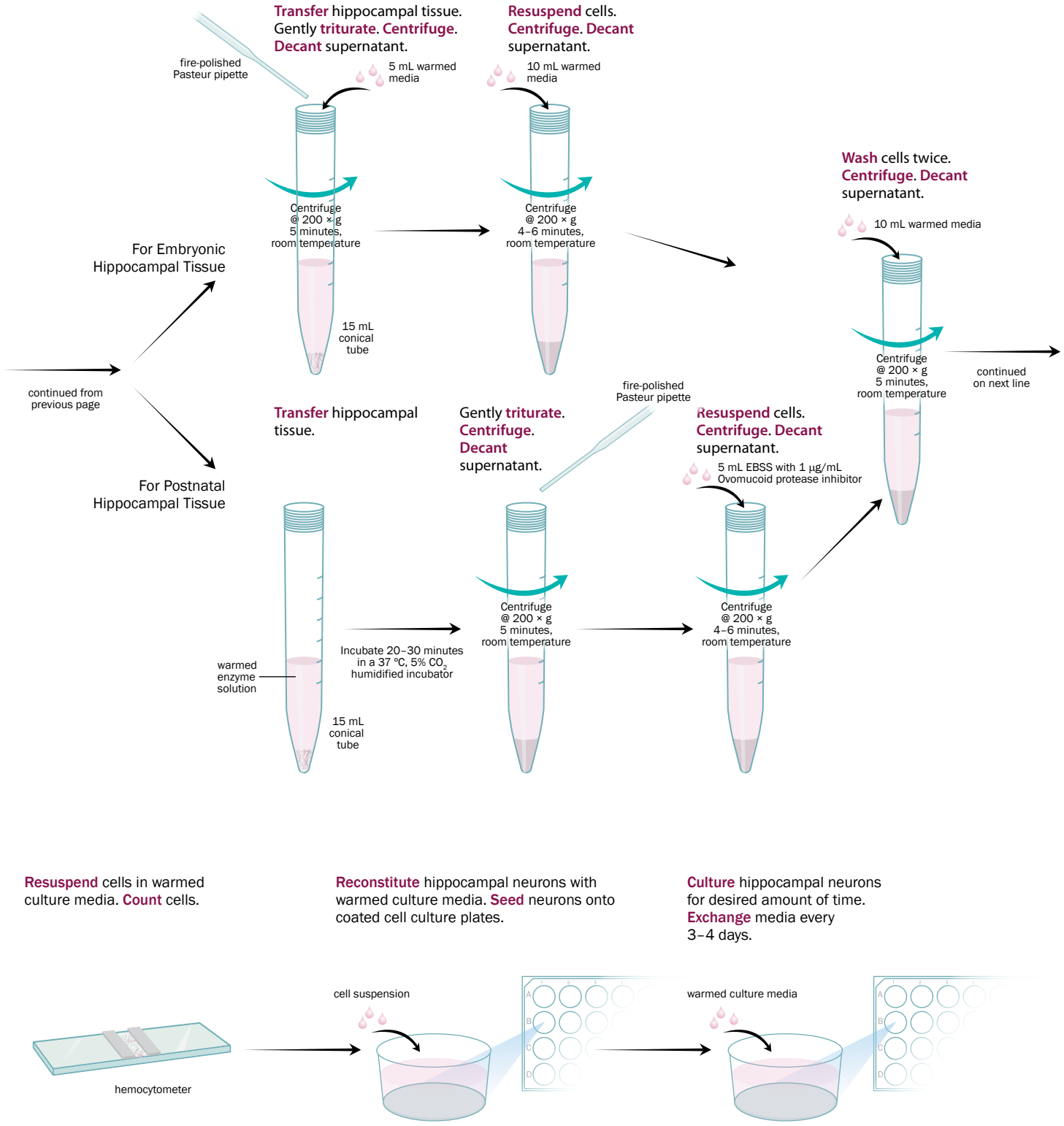
Protocol for Culturing Rat Hippocampal Neurons, continued



Protocol for Culturing Rat Microglia

Microglia are the resident immune cells of the central nervous system. They are important for maintaining homeostasis, monitoring tissue debris and pathogens, and eliminating or remodeling synapses. These cells are activated by neural injury and disease states, causing them to undergo morphological and proliferative changes, increase their phagocytic activity, and secrete proinflammatory molecules, which propagate neuroinflammation and neurodegenerative diseases. Utilizing primary microglia cell cultures provides a means to investigate the mechanisms by which these cells contribute to neurodegenerative diseases. This protocol provides step-by-step instructions for culturing microglia from isolated cortical tissue from 5–9 postnatal (P1–P2) rat pups. The protocol can be scaled up for more pups if needed.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of brain tissue can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- 70% ethanol
- Cultrex® Poly-L-Lysine (R&D Systems, Catalog # 3438-100-01)
- Deionized distilled H_2O, sterile (ddH_2O)
- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- Fetal Bovine Serum (FBS) - Premium Select (R&D Systems, Catalog # S11550)
- L-glutamine-penicillin-streptomycin solution (100x), or equivalent
- HBSS, Ca^{2+} and Mg^{2+} salts, no phenol red (R&D Systems, Catalog # B32450)
- Minimum Essential Medium Eagle (MEM)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)
- Trypsin 2.5% (10x, R&D Systems, Catalog # B81710)

Materials

- 5, 10, 25, and 50 mL pipettes
- 50 mL conical centrifuge tubes
- Cell culture flasks (T175), sterile
- Cell strainers, 40 µm (Fisher Scientific, Catalog # 22-363-547), or equivalent
- Ice
- P1–P2 rat pups
- Petri dishes, 100 × 20 mm
- Pipette tips
- Syringe filters, 0.22 µm (Cole-Parmer, Catalog # EW-81053-14), or equivalent

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Fine forceps, #5, straight
  - Forceps, #7, curved
  - Micro spatula
  - Surgical scissors, small
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Orbital shaker, 19 mm orbit diameter
- Pipette controller
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Co-culture Media

1. 1x L-glutamine-penicillin-streptomycin solution, 10% FBS in MEM

DNase I Stock Solution

1. 3 mg/mL DNase I in PBS
2. Filter using a 0.22 µm syringe filter to sterilize.

Note: Store at ≤ –20 °C for up to 2 years.

Procedure

Coating and Preparation of Cell Culture Flasks

Note: Preparation of the culture flasks should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-L-Lysine solution with PBS to a final concentration of 100 µg/mL.
2. Coat each T175 cell culture flask with 10 mL of the 100 µg/mL poly-L-lysine solution, and rock the flasks side-to-side.

Note: Other cell culture plates and flasks can be used to either scale up or down these cell cultures. See Table 2 (pg. 34) for the volume of 100 µg/mL poly-L-lysine that should be used for the different sized cell culture plates and flasks.

3. Incubate the flasks for at least 20 minutes in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-L-lysine solution. Wash the flasks two times with sterile ddH_2O. Aspirate to remove all liquids. Allow flasks to dry.

Note: Flasks can be stored at 2–8 °C for up to 2 months.

Dissection of Rat Cortical Tissue

Note: Soak dissection tools in 70% ethanol for at least 10 minutes to sterilize. Allow excess ethanol to drip off and place on a sterile, disinfected surface.

1. Add PBS to three 100 x 20 mm petri dishes and one 50 mL conical tube. Add 8 mL HBSS to a 50 mL conical tube. Place all on ice.
2. Decapitate P1–P2 rat pups at the head/neck junction using the small surgical scissors.
3. Place the heads in the chilled 50 mL conical tube containing PBS. Keep the tube on ice.
4. Transfer a rat pup head to a chilled 100 × 20 mm petri dish containing PBS.
5. Stabilize the dissociated head using the #7 curved forceps and #5 fine forceps. Moving caudal to rostral, cut through the skull with the small surgical scissors.

Note: Keep cuts shallow to avoid damaging the brain tissue.

6. Peel back the two halves of the separated skull.
7. Remove the whole brain from the head cavity using the micro spatula and place it in a second chilled 100 x 20 mm petri dish containing PBS. Repeat steps 4–7 for the remaining heads.
8. Using the #5 fine forceps and #7 curved forceps, separate the hemispheres along the median longitudinal fissure. Cut off and discard the brainstem, cerebellum, and olfactory bulbs. Place the remaining forebrains in the third chilled 100 × 20 mm petri dish.
9. Under a dissecting microscope, remove the meninges, midbrain, and hippocampus from each forebrain. Place the remaining cortical tissue in the Dissociation Tube, a chilled 50 mL conical tube containing 8 mL HBSS.

Dissociation and Culture of Rat Cortical Cells

Note: From this point forward, any opening of tubes/flasks that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

1. Warm the Co-Culture Media in 37 °C water bath. Warm the DNase I Stock Solution and 10x Trypsin solution to room temperature.
2. Add 200 µL of DNase I Stock Solution and 400 µL of 10x Trypsin to the cortical tissue in the Dissociation Tube. Using a 25 mL pipette, gently pipette the tissue up and down to break it into small pieces (~1 mm^2).

Note: The tissue can be cut into smaller pieces using a razor blade. Care should be taken to not over mince the tissue as this can increase the amount of fine debris.

3. Gently swirl the Dissociation Tube for 5 seconds and then incubate for 15 minutes in a 37 °C water bath.
4. Remove the Dissociation Tube from the water bath and let it sit undisturbed at room temperature for 5 minutes to let the tissue pieces settle.
5. Transfer all but 3 mL of the cell suspension to a new 50 mL conical tube, the Collection Tube. Add 2 mL of FBS and swirl to mix. Let sit at room temperature.

Note: Use plastic or siliconized glass to transfer the cell suspension as microglia can adhere to untreated glass surfaces.

6. Add 9 mL of HBSS, 200 µL of DNase I Stock Solution, and 400 µL of 10x Trypsin to the cortical tissue in the Dissociation Tube. Using a 10 mL pipette, gently pipette the tissue up and down to break it into smaller pieces.
7. Gently swirl the Dissociation Tube for 5 seconds. Incubate the tube in a 37 °C water bath for 15 minutes, agitating the contents of the tube by hand every 5 minutes.
8. Remove the Dissociation Tube from the water bath and let it sit undisturbed at room temperature for 5 minutes to let the tissue pieces settle.
9. Transfer the cell suspension to the Collection Tube, pooling it with the cell suspension and FBS solution from step 4. Save the cortical tissue.

Note: If a debris pellet has formed in the Collection Tube, remove it before pooling the cell suspension solutions.

10. Add 10 mL of PBS to the cortical tissue pieces in the Dissociation Tube. Swirl to mix and then let it sit undisturbed at room temperature for 5 minutes.
11. Transfer the PBS to the Collection Tube. Centrifuge the Collection Tube at 300 × g for 5 minutes at room temperature. Carefully discard the supernatant.

Note: Take care when decanting the supernatant as the cell pellet is not very firm at this stage.

12. Wash the cell pellet in the Collection Tube with 25 mL of PBS. Centrifuge at 300 × g for 5 minutes at room temperature. Decant the solution.
13. Resuspend the cells in 5 mL of warmed Co-Culture Media. Prepare two serial 1:5 dilutions of the cell suspension in 0.4% Trypan blue. Count the live cells.
14. Plate ~ 1.2 million cells in prepared T175 culture flasks containing 45 mL of warmed Co-Culture Media. Incubate in a 37° C, 5% CO_2 humidified incubator. This is Day 0 in the culture schedule (see table).

Culture Schedule

Note: Ideally, this cell culture protocol will result in astrocytes reaching confluency on days 9–10, allowing the microglia to be harvested 1–2 days later. However, the progression of the culture can vary based on a number of factors including debris level (more debris can cause the astrocytes to grow more slowly), speed of dissection, and serum variability. Thus, cultures should be monitored so the microglia can be harvested shortly after the astrocytes become confluent. Neuronal development is abundant about 3–4 days after astrocytes reach confluency, so, if microglia are harvested too late, neurons will shake off with the microglia. If the astrocytes are reaching confluency too soon or too late, plate fewer or more cortical cells, respectively, when setting up the mixed culture.

1. Prior to performing media changes, warm Co-Culture Media in a 37 °C water bath.
2. Follow the culture schedule.

Day 0	Plate ~1.2 million cells in prepared T175 culture flasks
Day 1	Cells separate and start to form processes.
Day 2	Astrocytes appear (large, flattened cells).
Day 4	Astrocytes are 2–6% confluent. Perform complete media change with warmed Co-Culture Media.
Day 7	Astrocytes are 60–80% confluent. Perform complete media change with warmed Co-Culture Media.
Day 9–10	Astrocytes reach 100% confluence
Day 11–12	Harvest microglia

Note: Do not feed the culture after Day 7 because microglia, and not neurons, will thrive under conditions of nutrient depletion.

Harvesting Rat Microglia

1. Place T175 flasks on shaker inside a humidified 37° C, 5% CO_2 incubator. Shake for 2 hours at 180 rpm.
2. Collect the Co-Culture Media from a flask and pass it through a 40 µm cell strainer into a 50 mL conical tube. Repeat for all flasks.
3. Centrifuge at 300 × g for 5 minutes at room temperature. Discard the supernatant.
4. Resuspend microglia in desired media. Pool cells if needed.

Note: Protocol will result in about 2–6 million microglia per T175 flask.

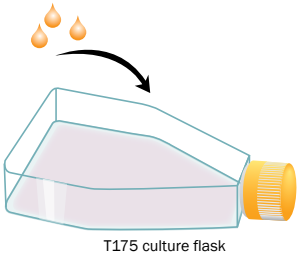
References

1. Ni, M. and M. Aschner (2010). Curr. Protoc. Toxicol. Unit **43**:12.17:1–16.

Protocol for Culturing Rat Microglia

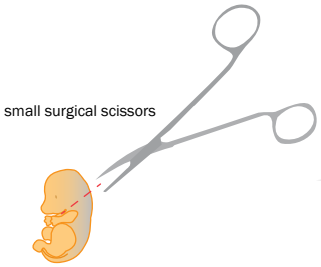
Day 1

Prepare T175 cell culture flasks by coating with Poly-L-Lysine.



Day 2

Decapitate P1–2 rat pups.



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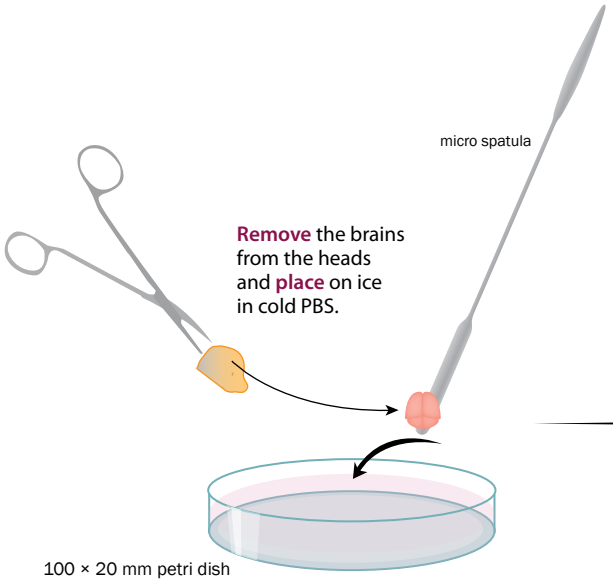
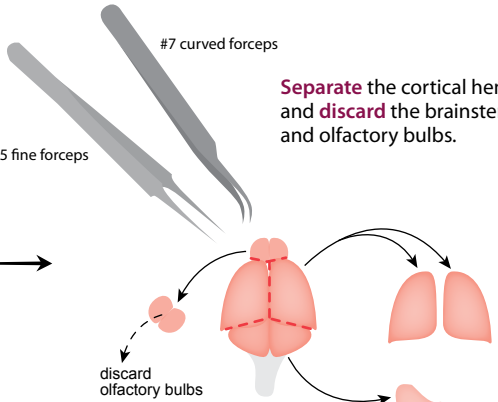


Remove the brains from the heads and **place** on ice in cold PBS.



Separate the cortical hemispheres and **discard** the brainstem, cerebellum, and olfactory bulbs.

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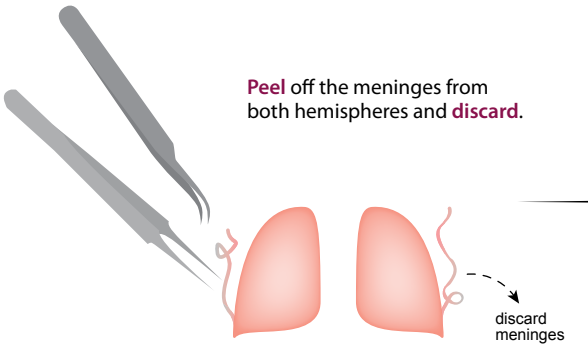
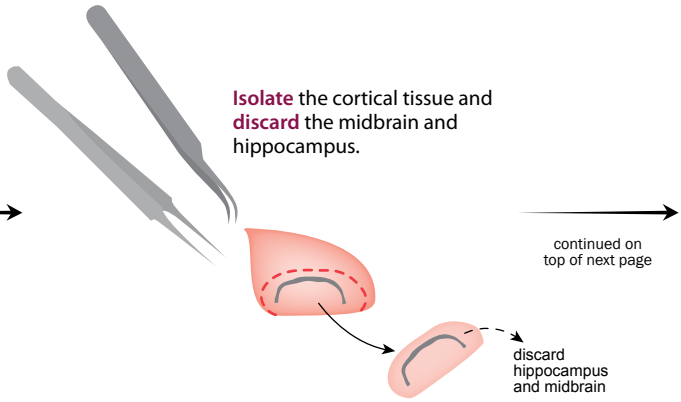


Peel off the meninges from both hemispheres and **discard**.



Isolate the cortical tissue and **discard** the midbrain and hippocampus.

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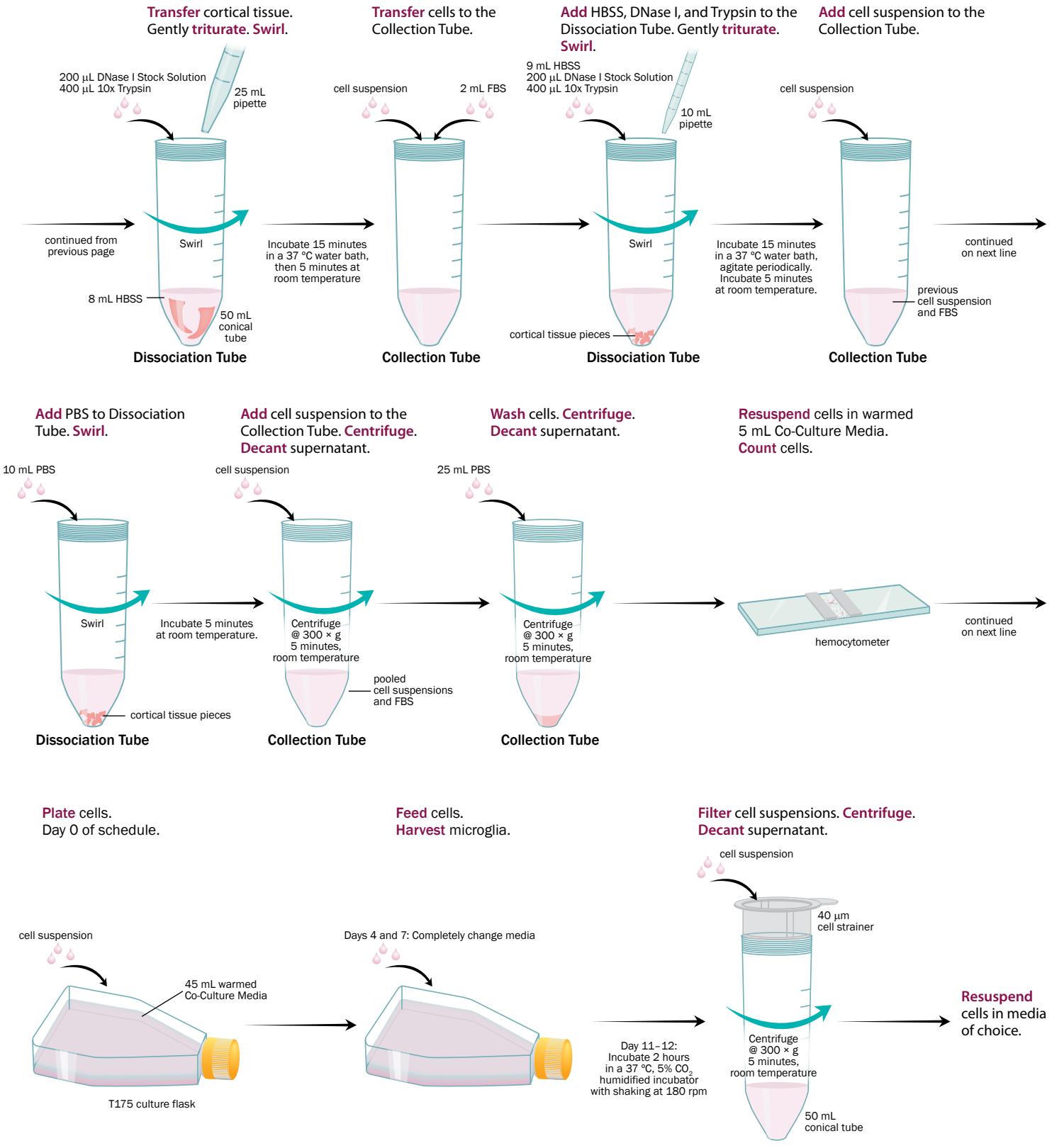
Protocol for Culturing Rat Microglia, continued



Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 CD-1 mice, can be grown in a monolayer or as neurospheres. The neurosphere culture system described here is useful for investigating processes involved in neural stem cell proliferation and differentiation. Neurospheres are cultured in defined, serum-free media enabling researchers to interrogate stem cell growth and differentiation by modulating cues in the extrinsic environment. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. All steps should be conducted within a laminar flow cell culture hood, and all reagents and materials used should be sterile.

Caution: The mouse cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO, and the media used in this protocol contains trace amounts of Transferrin. The Transferrin was purified from donor plasma and tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigens.

Supplies Required

Reagents

- Acetic acid
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (Millipore Sigma, Catalog # 810683), or equivalent
- Deionized H_2O (dH_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- L-Glutamine, powder (R&D Systems, Catalog # R90010
- Mouse Cortical Stem Cells (R&D Systems, Catalog # NSC002)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$, powder (R&D Systems, Catalog # R39150)
- DPBS, no Ca^{2+} and Mg^{2+} salts, no phenol red (R&D Systems, Catalog # B30250)
- Recombinant Human EGF Protein (R&D Systems, Catalog # 236-EG)
- Recombinant Human FGF basic/FGF2/bFGF Protein (bFGF, R&D Systems, Catalog # 3718-FB or 4114-TC)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-Streptomycin 10/10 (100x, R&D Systems, Catalog # B21210)

Materials

- 0.2 µm, 500 mL, sterile filter unit
- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (6-well), sterile (Corning™ # 3516), or equivalent
- Pipette tips
- Serological pipettes

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Laminar flow cell culture hood
- Inverted microscope
- Pipette pump
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with dH_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
L-Glutamine	0.0365 g
$NaHCO_3$	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 0.2 µm filter unit.

Note: Penicillin-Streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

FGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in DPBS to the Human bFGF vial to make a 20 µg/mL stock solution.

EGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in 10 mM acetic acid to the Human EGF vial to make a 20 µg/mL stock solution.

Note: Aliquot and store protein stock solutions at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Thawing Cryopreserved Cells

Note: Review the following protocol in detail before thawing the cells.

1. Add the FGF and EGF stock solutions to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.
2. Add 20 mL of warmed Completed NSC Base Media with mitogens to a sterile 50 mL conical tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.

3. Remove the cryovial containing frozen Mouse Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
5. Carefully aspirate off 95% of the supernatant. Resuspend the cells in 10 mL of warmed Completed NSC Base Media with mitogens by gently pipetting.

Note: Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw slowly in the DMSO will dramatically reduce viability. Around 90% cell viability is expected from the freshly thawed cells when the appropriate thawing procedure is followed.

6. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells. Seed cells at a density according to the appropriate expansion protocol.

Cell Expansion

Note: Use serological pipettes to transfer and remove solutions.

1. Warm an appropriate amount of Completed NSC Base media. If needed, add EGF and FGF stock solutions to the media at a final concentration of 20 ng/mL.
2. Seed approximately 2 × 10^5 Mouse Cortical Stem Cells in 5 mL of warmed Completed NSC Base Media supplemented with mitogens per well in a 6-well cell culture plate.
3. Keep cells in a 37 °C, 5% CO_2 humidified incubator.
4. Add fresh EGF and FGF stock solutions at a final concentration of 20 ng/mL each day to the media. Every fourth day, replace the media according to the number of neurospheres present.

   For 50 neurospheres or more:

   a. Transfer the media containing the neurospheres to a 15 mL conical tube.
   b. Centrifuge at 100 × g for 5 minutes at room temperature. Decant the media.
   c. Gently resuspend the pellet using a small quantity of fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL bFGF.
   d. Add the neurosphere suspension to one well of a 6-well cell culture plate that contains 5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL bFGF.

   For less than 50 neurospheres:

   a. Transfer the neurospheres directly into one well of a 6-well cell culture plate that contains 2.5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL bFGF.

Note: Do not discard the conditioned media. When there are fewer neurospheres, conditioned media is required. Only half of the media is replaced with fresh, warmed Completed NSC Base Media with mitogens.

   b. Add 2.5 mL of the conditioned media to the well.

5. Pass the cells at 5–7 days, or when the neurospheres have a dark clump inside, or ruffling on the outside of the neurosphere, according to the procedure described below.

Passaging Cells

1. Warm an appropriate amount of Completed NSC Base Media. If needed, add EGF and FGF stock solutions to the media at a final concentration of 20 ng/mL.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube. Do not dislodge attached neurospheres for passage.
3. Centrifuge for 5 minutes at 100 × g at room temperature. Carefully aspirate off 95% of the supernatant.
4. Partially dissociate the pelleted neurospheres by pipetting the remaining media up and down 20 times, being careful not to create bubbles in the suspension.

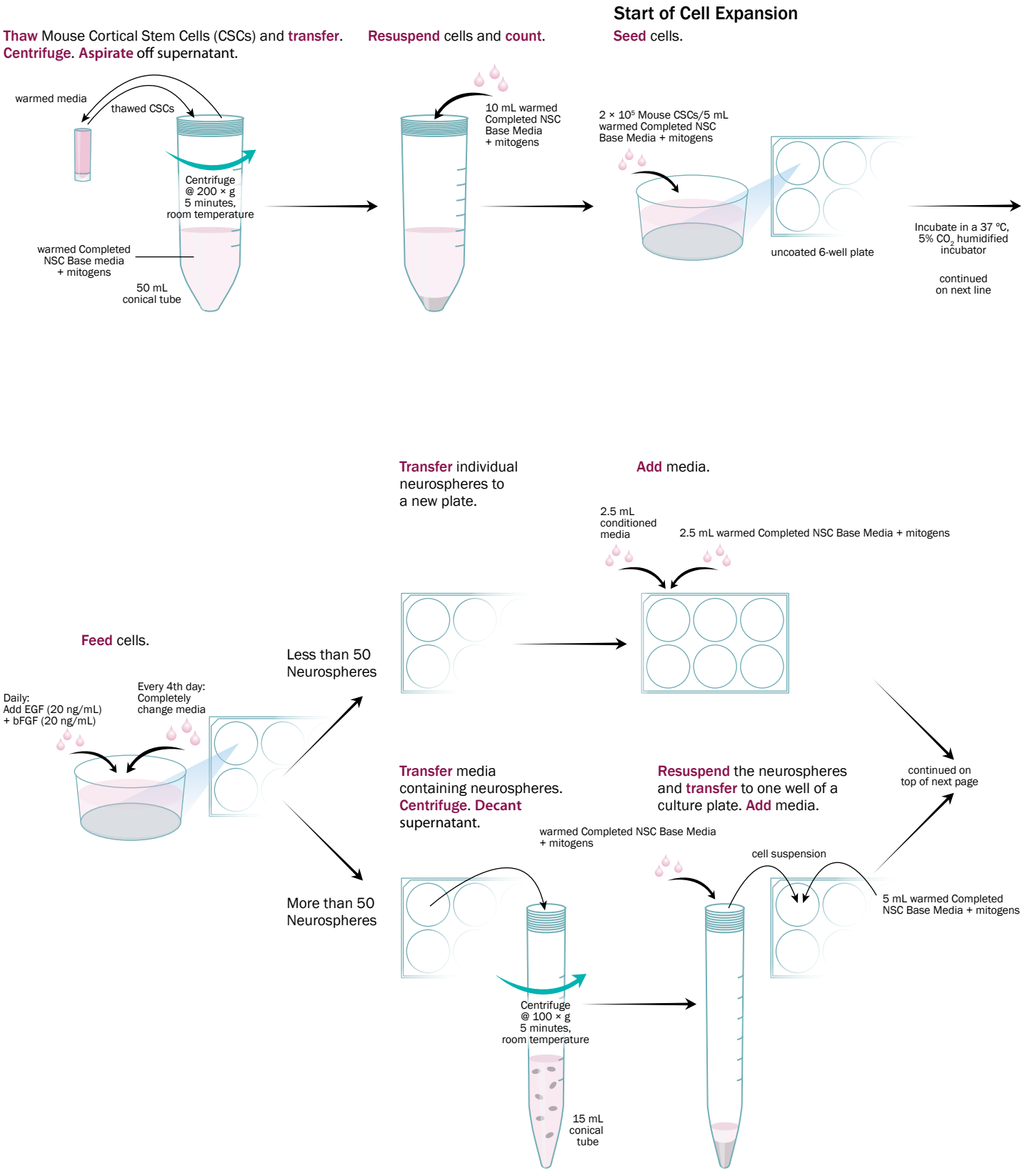
Note: For optimal dissociation of the neurospheres, it is recommended that a P200 pipette be used.

5. At the initial passages 1 and 2, add 5 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Transfer the final neurosphere suspension into one well of a 6-well cell culture plate. Repeat from step 3 in the Cell Expansion section (see above).
6. After passage 2, add 10 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Split the final neurosphere suspension into two wells of a 6-well cell culture plate (5 mL of neurosphere suspension/well). Repeat from step 3 in the Cell Expansion section (see above).

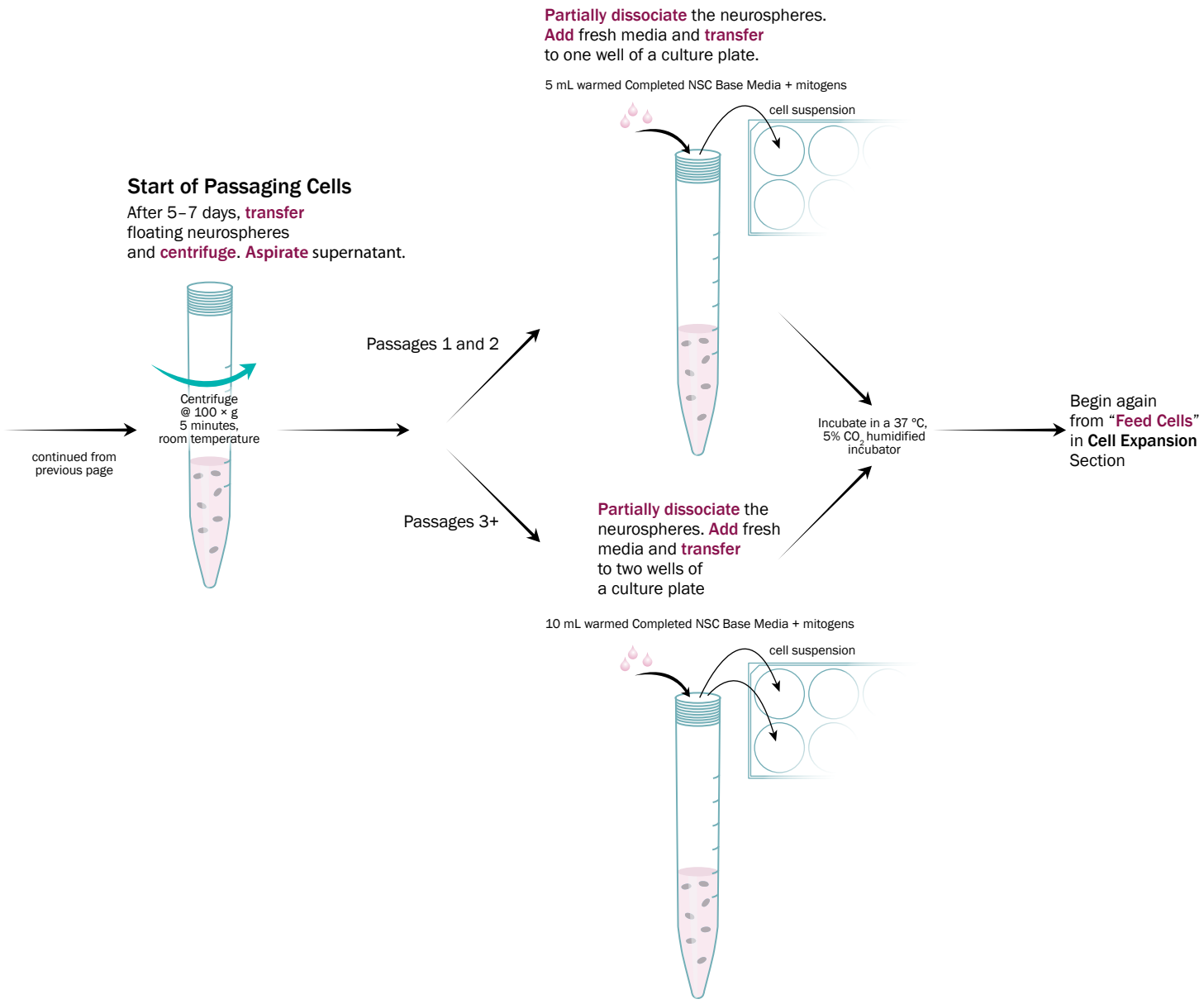
References

1. Johe, K.K. *et al.* (1996) Genes Dev. **10**:3129.
2. Kim, J.H. *et al.* (2003) Methods Enzymol. **365**:303.
3. Tropepe, V. *et al.* (1999) Dev. Biol. **208**:166.
4. T.J. Kilpatrick and P.F. Bartlett (1993) Neuron **10**:255.

Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System



Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System, continued



Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 Sprague-Dawley rats, can be grown in a monolayer or as neurospheres. The neurosphere culture system described here is useful for investigating processes involved in neural stem cell proliferation and differentiation. Neurospheres are cultured in defined, serum-free media enabling researchers to interrogate stem cell growth and differentiation by modulating cues in the extrinsic environment. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

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Caution: The rat cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO, and the media used in this protocol contains trace amounts of Transferrin. The Transferrin was purified from donor plasma and tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigens.

Supplies Required

Reagents

- Acetic acid
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (Millipore Sigma, Catalog # 810683), or equivalent
- Deionized H_2O (dH_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- L-Glutamine, powder, (R&D Systems, Catalog # R90010)
- Rat Cortical Stem Cells (R&D Systems, Catalog # NSC001)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$, powder, (R&D Systems, Catalog # R39150)
- DPBS, no Ca^{2+} and Mg^{2+} salts, no phenol red (R&D Systems, Catalog # B30250)
- Recombinant Human EGF Protein (R&D Systems, Catalog # 236-EG)
- Recombinant Human FGF basic/FGF2/bFGF Protein (bFGF, R&D Systems, Catalog # 3718-FB or 4114-TC)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-Streptomycin 10/10 (100x, R&D Systems, Catalog # B21210)

Materials

- 0.2 µm, 500 mL, sterile filter unit
- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (6-well), sterile (Corning™ # 3516), or equivalent
- Pipette tips
- Serological pipettes

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Laminar flow cell culture hood
- Inverted microscope
- Pipette pump
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with dH_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
L-Glutamine	0.0365 g
$NaHCO_3$	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 0.2 µm filter unit.

Note: Penicillin-Streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

FGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in DPBS to the Human bFGF vial to make a 20 µg/mL stock solution.

EGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in 10 mM acetic acid to the Human EGF vial to make a 20 µg/mL stock solution.

Note: Aliquot and store protein stock solutions at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Thawing Cryopreserved Cells

Note: Review the following protocol in detail before thawing the cells.

1. Add the FGF and EGF stock solutions to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.
2. Add 20 mL of warmed Completed NSC Base Media with mitogens to a sterile 50 mL conical tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.

3. Remove the cryovial containing frozen Rat Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
5. Carefully aspirate off 95% of the supernatant. Resuspend the cells in 10 mL of warmed Completed NSC Base Media with mitogens by gently pipetting.

Note: Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw slowly in the DMSO will dramatically reduce viability. Around 90% cell viability is expected from the freshly thawed cells when the appropriate thawing procedure is followed.

6. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells. Seed cells at a density according to the appropriate expansion protocol.

Cell Expansion

Note: Use serological pipettes to transfer and remove solutions.

1. Warm an appropriate amount of Completed NSC Base media. If needed, add EGF and FGF stock solutions to the media at a final concentration of 20 ng/mL.
2. Seed approximately 1 × 10^5 Rat Cortical Stem Cells in 5 mL of warmed Completed NSC Base Media supplemented with mitogens per well in a 6-well cell culture plate.
3. Keep cells in a 37 °C, 5% CO_2 humidified incubator.
4. Add fresh EGF and FGF stock solutions to a final concentration of 20 ng/mL each day to the media. Every fourth day, replace the media according to the number of neurospheres present.

For 50 neurospheres or more:

a. Transfer the media containing the neurospheres to a 15 mL conical tube.
b. Centrifuge at 100 × g for 5 minutes at room temperature. Decant the media.
c. Gently resuspend the pellet using a small quantity of fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL bFGF.
d. Add the neurosphere suspension into one well of a 6-well cell culture plate that contains 5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL bFGF.

For less than 50 neurospheres:

a. Transfer the neurospheres directly into one well of a 6-well cell culture plate that contains 2.5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL bFGF.

Note: Do not discard the conditioned media. When there are fewer neurospheres, conditioned media is required. Only half of the media is replaced with fresh, warmed Completed NSC Base Media with mitogens

b. Add 2.5 mL of the conditioned media to the well.

5. Pass the cells at 5–7 days, or when the neurospheres have a dark clump inside, or ruffling on the outside of the neurosphere, according to the procedure described below.

Passaging Cells

1. Warm an appropriate amount of Completed NSC Base Media. If needed, add EGF and FGF stock solutions to the media at a final concentration of 20 ng/mL.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube. Do not dislodge attached neurospheres for passage.
3. Centrifuge for 5 minutes at 100 × g at room temperature. Carefully aspirate off 95% of the supernatant
4. Partially dissociate the pelleted neurospheres by pipetting the remaining media up and down 20 times, being careful not to create bubbles in the suspension.

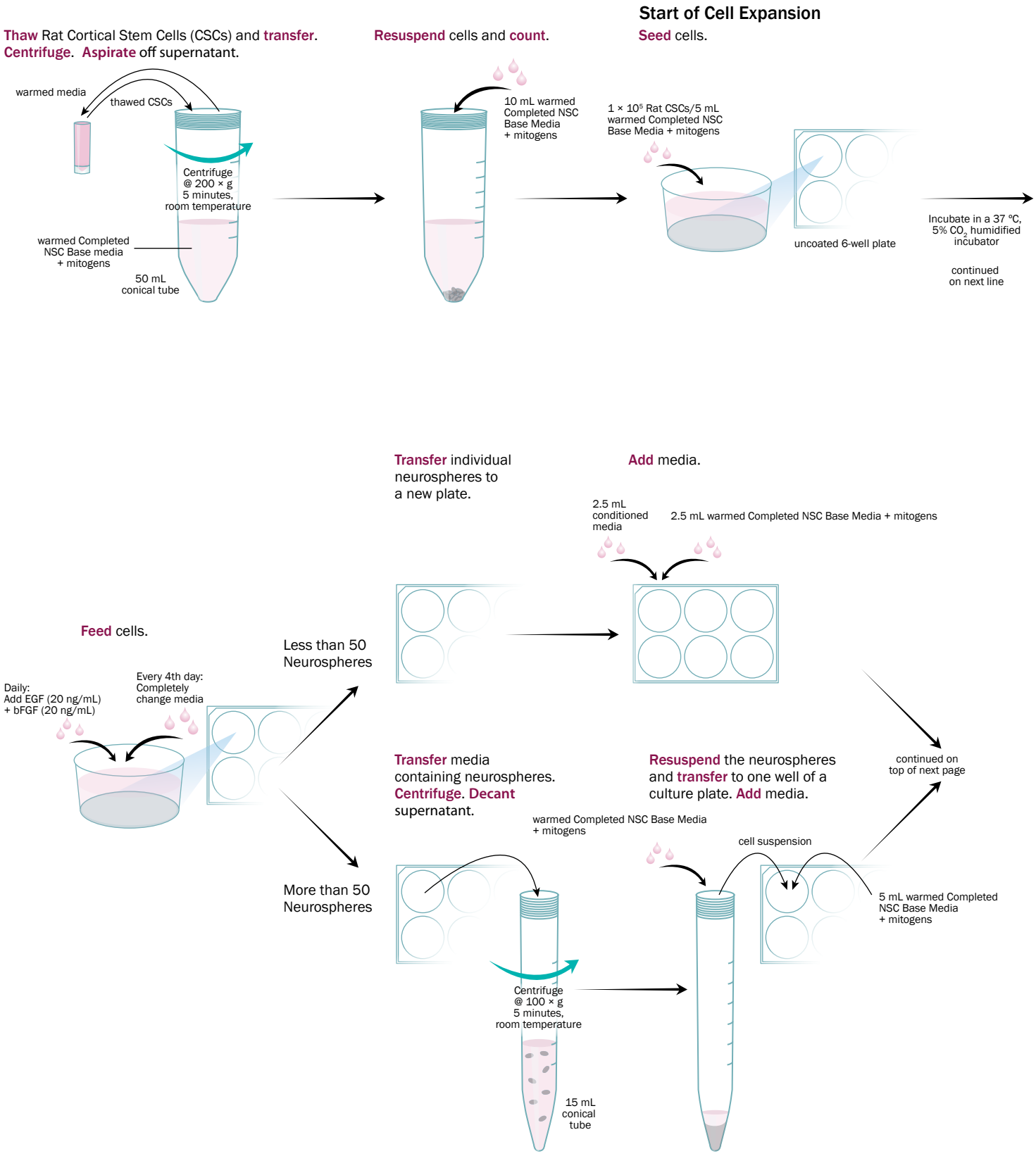
Note: For optimal dissociation of the neurospheres, it is recommended that a P200 pipette be used.

5. At the initial passages 1 and 2, add 5 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Transfer the final neurosphere suspension into one well of a 6-well cell culture plate. Repeat from step 3 in the Cell Expansion section (see above).
6. After passage 2, add 10 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Split the final neurosphere suspension into two wells of a 6-well cell culture plate (5 mL of neurosphere suspension/well). Repeat from step 3 in the Cell Expansion section (see above).

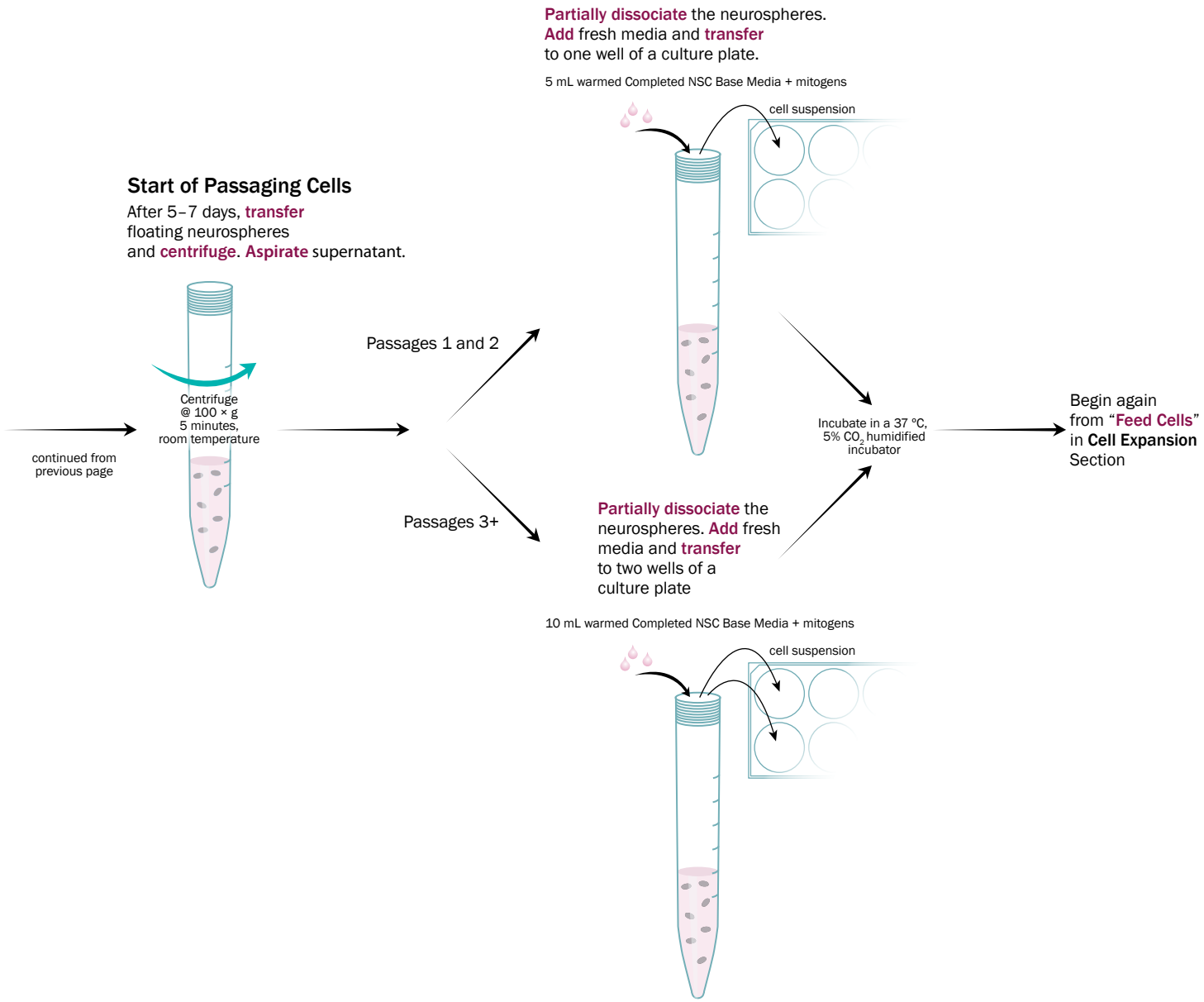
References

1. Johe, K.K. *et al.* (1996) Genes Dev. **10**:3129.
2. Kim, J.H. *et al.* (2003) Methods Enzymol. **365**:303.

Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System



Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System, continued



Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Monolayer System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 Sprague-Dawley rats can be grown in a monolayer, as described here, or as neurospheres. Monolayer cultures provide a more homogenous population of undifferentiated precursor cells and help to inhibit spontaneous differentiation. Additionally, cells grown in a monolayer can be directly monitored and investigated using methods such as functional assays and immunocyto-chemistry. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. All steps should be conducted within a laminar flow cell culture hood, and all reagents and materials used should be sterile.

Caution: The rat cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO. The Transferrin was purified from donor plasma and tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigens.

Supplies Required

Reagents

- Bovine Fibronectin Protein (R&D Systems, Catalog # 1030-FN)
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (Millipore Sigma, Catalog # 810683), or equivalent
- Cultrex® Poly-L-Ornithine Solution (0.1 mg/mL, R&D Systems, Catalog # 3436-100-01)
- Deionized H_2O (dH_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- L-Glutamine, powder, (R&D Systems, Catalog # R90010)
- HBSS, no Ca^{2+}, no Mg^{2+}, no phenol red (10x, ThermoFisher Scientific, Catalog # 14185052)
- HEPES (Ultra Pure), powder, (R&D Systems, Catalog # R35150)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$, powder, (R&D Systems, Catalog # R39150)
- DPBS, no Ca^{2+} and Mg^{2+} salts, no phenol red, (R&D Systems, Catalog # B30250)
- Rat Cortical Stem Cells (R&D Systems, Catalog # NSC001)
- Recombinant Human FGF basic/FGF2/bFGF Protein (bFGF, R&D Systems, Catalog # 3718-FB)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-Streptomycin 10/10 (100x, R&D Systems, Catalog # B21210)

Materials

- 0.2 µm, 500 mL, sterile filter units
- 0.2 µm, 1000 mL, sterile filter units
- 10 cm tissue culture dishes, sterile
- 50 mL conical centrifuge tubes
- Plastic cell scraper, sterile
- Pipette tips

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Inverted microscope
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with dH_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
L-Glutamine	0.0365 g
$NaHCO_3$	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 500 mL, 0.2 µm filter unit.

Note: Penicillin-Streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

Buffered HBSS (1x)

1. Add 100 mL of HBSS (10x) and 3.9 g HEPES to 900 mL of dH_2O.
2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 1000 mL, 0.2 µm filter unit.

Note: Buffered HBSS can be stored at room temperature for up to 6 months.

FGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA to the Human bFGF vial to make a 20 µg/mL stock solution.

Note: Aliquot and store the FGF stock solution at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Culture Dish Preparation

1. Dilute the 0.1 mg/mL Cultrex® Poly-L-Ornithine Solution with sterile DPBS to a final concentration of 15 µg/mL.

Note: Prepare this solution fresh as needed.

2. Add 10 mL of the 15 µg/mL poly-L-ornithine solution to each 10 cm tissue culture dish. Incubate the dishes overnight in a 37 °C, 5% CO_2 humidified incubator.
3. Discard the poly-L-ornithine solution. Wash each tissue culture dish three times with 10 mL of sterile DPBS. Add 10 mL of sterile DPBS to each dish. Incubate the tissue culture dishes overnight in a 37 °C, 5% CO_2 humidified incubator.
4. Allow the vial of bovine Fibronectin protein to warm to room temperature without agitation. Add sterile DPBS to the bovine Fibronectin protein to make a final concentration of 1 µg/mL. Gently invert the vial to mix.

Note: Prepare this solution fresh as needed.

5. Discard the DPBS from each of the poly-L-ornithine-coated dishes. Wash each tissue culture dish once with 10 mL of sterile DPBS.
6. Add 10 mL of the 1 µg/mL bovine Fibronectin solution to each tissue culture dish. Incubate the dishes for 3–30 hours in a 37 °C, 5% CO_2 humidified incubator.
7. Prior to use, discard the bovine Fibronectin solution and wash each tissue culture dish once with 10 mL of sterile DPBS.

Thawing Cryopreserved Cells

Note: Review the following section in detail before thawing the cells.

1. Add the FGF stock solution to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.
2. Add 20 mL of warmed Completed NSC Base Media with bFGF to a sterile 50 mL conical centrifuge tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.
3. Remove the cryovial containing frozen Rat Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue and count the live cells.
5. Seed cells at a density according to the expansion protocol described below.

Cell Expansion

1. Seed 1–1.5 × 10^6 Rat Cortical Stem Cells in 10 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL of bFGF on a poly-L-ornithine/Fibronectin-coated tissue culture dish.
2. Incubate the tissue culture plates for 3 hours to overnight in a 37 °C, 5% CO_2 humidified incubator. After the cells become adherent, replace the media with fresh, warmed Completed NSC Base Media supplemented with 20 ng/mL bFGF and return the plates to the 37 °C, 5% CO_2 humidified incubator.
3. After 24 hours, add 10 µL of the 20 µg/mL FGF stock solution to the culture.
4. Each day, supplement the media in the tissue culture plates with 10 µL of the 20 µg/mL FGF stock solution. Every second day, replace the media with fresh, warmed Completed NSC Base Media.
5. When the cultures reach 60–70% confluency (~ 4 days after initial plating), passage the cells according to the procedure described below.

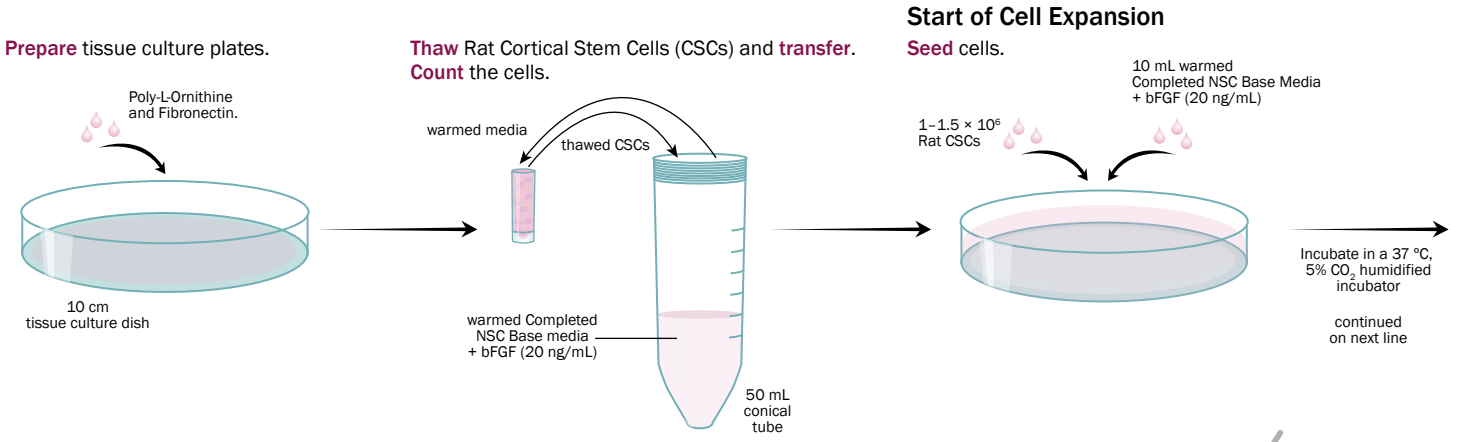
Passaging Cells

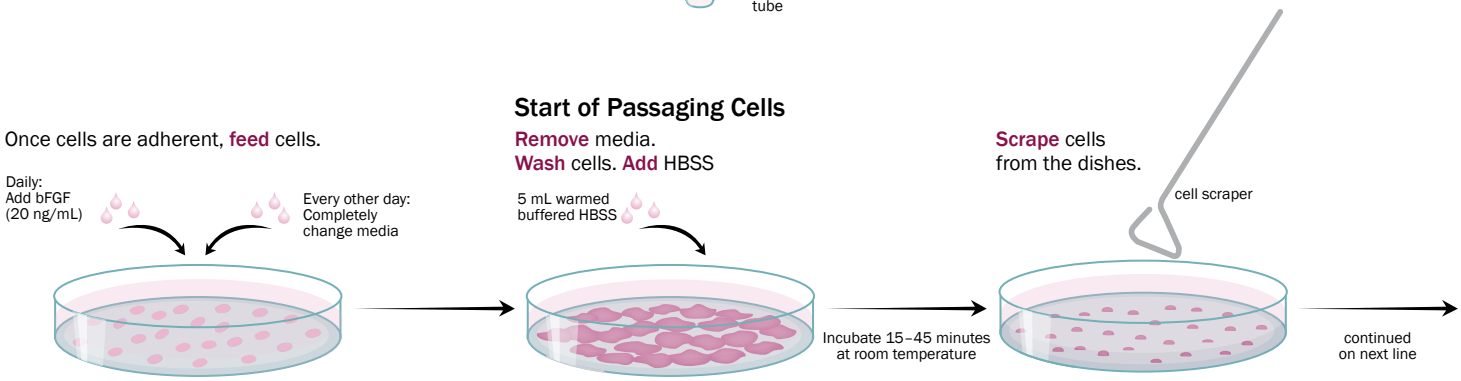
1. Warm the buffered HBSS (1x) and Completed NSC Base Media supplemented with 20 ng/mL bFGF in a 37 °C water bath.
2. Remove the media from the cells. Wash the cells once with 10 mL of warmed buffered HBSS (1x).
3. Add 5 mL of warmed buffered HBSS (1x) to the tissue culture plates. Incubate the plates for 15–45 minutes at room temperature until the cells round up.

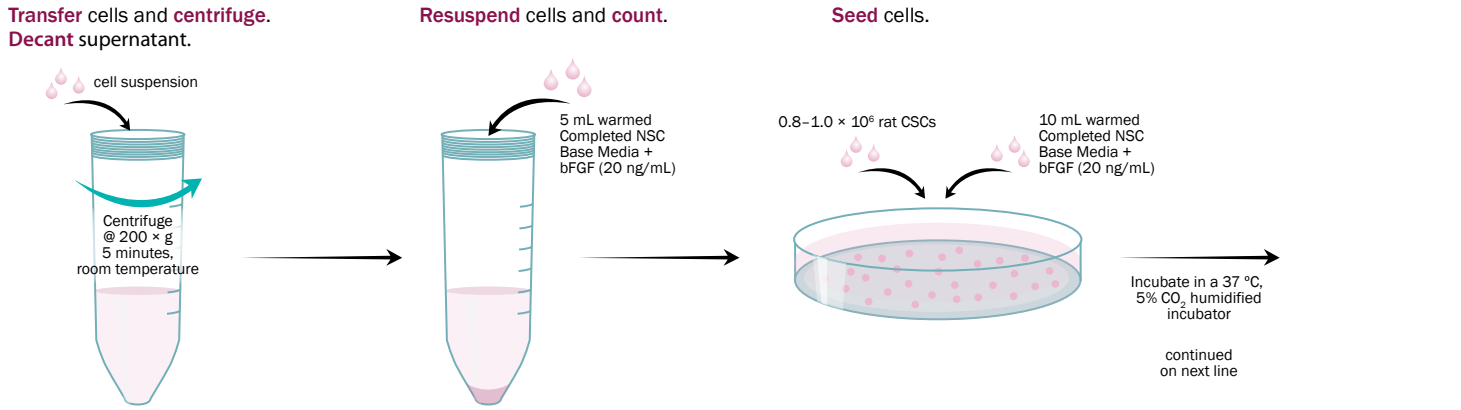
Note: Check the cultures frequently.

4. Scrape the cells from the tissue culture plates with a sterile, hard plastic cell scraper. Transfer the cells to a sterile 50 mL conical tube. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution from the pelleted cells.
5. Add 5 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL bFGF to the pelleted cells. Using a 5 mL pipette, resuspend the cells by slowly pipetting up and down (~5 times).
6. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
7. Seed 0.8–1.0 × 10^6 viable cells on a poly-L-ornithine/Fibronectin-coated plate in 10 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL bFGF.
8. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator. Repeat steps 4 and 5 in the Cell Expansion section (see above). Passage the cells after 3 days or when cells reach 70% confluency.

Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Monolayer System







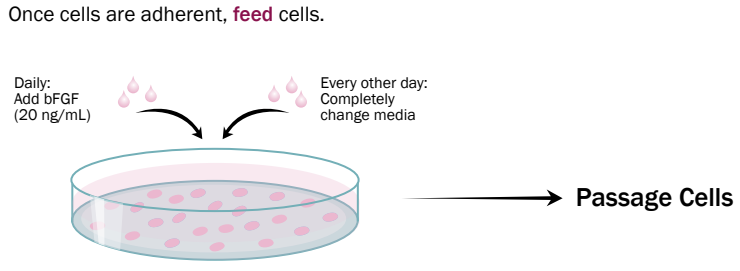


Table 1. Recommended Seeding Densities for Cortical/Hippocampal Neuron Cultures

Culture Dishes/Chamber Slides	Media Volume (mL)/Well	Total Number of Cells Required to Seed Each Well		
		Low Density (2.5×10^4 cells/cm^2)	Medium Density (5.0×10^4 cells/cm^2)	High Density (2.5×10^5 cells/cm^2)
6-well plate	2.0	2×10^5	5×10^5	2×10^6
12-well plate	0.8	1×10^5	2×10^5	1×10^6
24-well plate	0.5	5×10^4	1×10^5	5×10^5
48-well plate	0.3	2.5×10^4	5×10^4	2.5×10^5
96-well plate	0.1	5×10^3	2×10^4	5×10^4
4-well µ-slide	0.5	5×10^4	1×10^5	5×10^5
8-well µ-slide	0.3	2×10^4	4×10^4	2×10^5

Table 2. Poly-L-Lysine Coating Volumes for Different Sized Cell Culture Plates and Flasks

Culture Plate	Coating Volume (µL/well)
6-well plate	1000
12-well plate	500
24-well plate	200
48-well plate	100
96-well plate	50

Culture Flask	Coating Volume (mL)
T175 flask	10
T75 flask	5
T25 flask	2.5

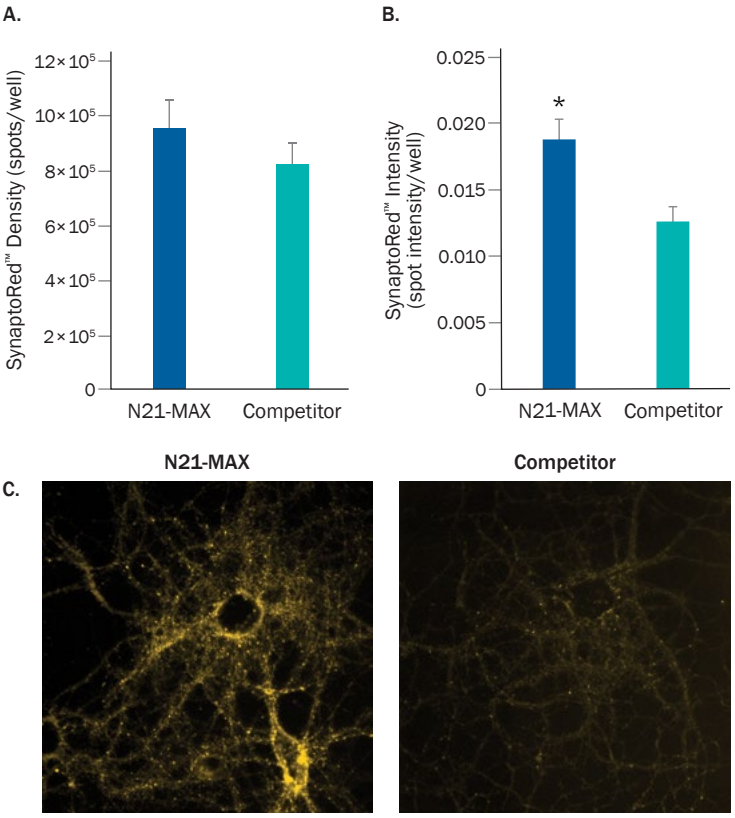
Neural Cell Culturing Products

R&D Systems™ and Tocris™ Cell Culture Products

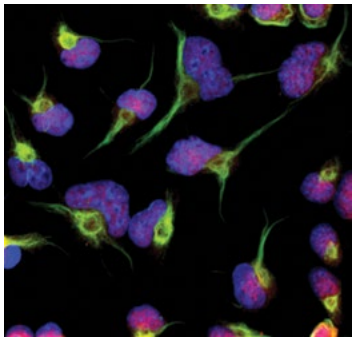
Make Your Cell Culture Workflow Easier

with products designed for reliability and efficiency.

- **Culture with Confidence.**
  From cell culture media and supplements to adhesion substrates and complete culture kits, our tools for neural cell culture are designed to ensure that your *in vitro* and *ex vivo* experiments perform optimally.
- **Cell Culture Basics by the Best.**
  We offer media, serum, chemicals, buffers, solvents, and antibiotics with the same high level of quality that we put into the rest of our portfolio.
- **Optimized Kits and Media Supplements.**
  With high-quality growth factors and cytokines at our disposal, we've designed kits and media for the growth and differentiation of immune cells, brain cells, and stem cells.



N21-MAX Media Supplement Enhances Synaptic Development. E18 rat hippocampal neurons were grown for 19 days *in vitro* in media supplemented with either N21-MAX Media Supplement (R&D Systems, Catalog # AR008) or the neural media supplement from the most widely-used competitor. Cells were incubated with the synaptic vesicle dye SynaptoRed™ C2 (Tocris, Catalog # 5118), for 1 minute prior to depolarization with KCl. Cells were imaged for SynaptoRed™ C2-positive synaptic puncta. (**A**) Quantification of SynaptoRed™ C2-positive puncta shows that neurons grown in the N21-MAX Media Supplement have an increased number of synaptic puncta compared to the competitor media. (**B**) Quantification of dye intensity shows that neurons grown in the N21-MAX Media Supplement have more robust synaptic activity than neurons cultured in the competitor media. (**C**) Representative images of SynaptoRed™ C2 staining in neurons cultured in the N21-MAX Media Supplement or competitor media.



N-2 MAX Media Supplement Provides Optimal Growth Conditions for Neural Progenitor Cell Expansion. Human neural progenitor cells were maintained in culture with N-2 MAX Media Supplement (Catalog # AR009). The cells were then stained for SOX1 expression using a Goat Anti-Human/Mouse/Rat SOX1 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF3369) followed by a NorthernLights™ (NL) 557-Conjugated Donkey Anti-Goat IgG Secondary Antibody (Catalog # NL001; red). The cells were also stained for Nestin expression using a Mouse Anti-Mouse/Rat Nestin Monoclonal Antibody (Catalog # MAB2736) followed by a NL493-Conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL009; green). The cells were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

Learn more | rndsystems.com/neuralculture

R&D Systems™ Cell Culture Media

Product	Catalog #s	
	With Phenol Red	Without Phenol Red
*DMEM, high glucose, 25 mM HEPES, L-glutamine, no sodium pyruvate	M22550	M19750
*DMEM, high glucose, 25 mM HEPES, L-glutamine, sodium pyruvate	M22650	M18550
DMEM, high glucose, 25 mM HEPES, no glutamine, no sodium pyruvate	M19550	M19650
DMEM, high glucose, 25 mM HEPES, no glutamine, sodium pyruvate	M18250	M18350
*DMEM, high glucose, L-glutamine, no sodium pyruvate	M22250	M12950
*DMEM, high glucose, L-glutamine, sodium pyruvate	M22450	M18650
DMEM, high glucose, no glutamine, sodium pyruvate	M12450	M18450
DMEM, high glucose, no glutamine, no sodium pyruvate	M22850	M22950
*DMEM, low glucose, 25 mM HEPES, L-glutamine, no sodium pyruvate	M17050	M17150
*DMEM, low glucose, 25 mM HEPES, L-glutamine, sodium pyruvate	M22350	M12750
DMEM, low glucose, 25 mM HEPES, no glutamine, no sodium pyruvate	M13850	M13950
DMEM, low glucose, 25 mM HEPES, no glutamine, sodium pyruvate	M12350	M12550
*DMEM, low glucose, L-glutamine, no sodium pyruvate	M17250	M17350
*DMEM, low glucose, L-glutamine, sodium pyruvate	M22150	M12850
DMEM, low glucose, no glutamine, no sodium pyruvate	M14050	M12250
DMEM, low glucose, no glutamine, sodium pyruvate	M12150	M12650
*DMEM/F-12, 15 mM HEPES, L-glutamine	M23250	M23850
DMEM/F-12, 15 mM HEPES, no glutamine	M23050	M23550
*DMEM/F-12, L-glutamine	M23150	M23650
DMEM/F-12, no glutamine	M23350	M23750
*F-12, 25 mM HEPES, L-glutamine	M25250	M15450
F-12, 25 mM HEPES, no glutamine	M15050	M15250
*F-12, L-glutamine	M25150	M15350

Product	Catalog #s	
	With Phenol Red	Without Phenol Red
F-12, no L-glutamine	M15150	M25350
*L-15 (Leibovitz), 25 mM HEPES, L-glutamine	M40250	M40450
L-15 (Leibovitz), 25 mM HEPES, no glutamine	M40050	M40350
*L-15 (Leibovitz), L-glutamine	M40150	M40750
L-15 (Leibovitz), no glutamine	M40550	M40650
*MEM (Earle's salts), 25 mM HEPES, L-glutamine	M29650	M37350
MEM (Earle's salts), 25 mM HEPES, no glutamine	M39650	M37250
*MEM (Earle's salts), 25 mM HEPES, non-essential amino acids, L-glutamine	M29750	M36250
MEM (Earle's salts), 25 mM HEPES, non-essential amino acids, no glutamine	M29950	M32250
*MEM (Earle's salts), L-glutamine	M29150	M37450
MEM (Earle's salts), no glutamine	M29050	M29450
*MEM (Earle's salts), non-essential amino acids, L-glutamine	M29250	M36350
MEM (Earle's salts), non-essential amino acids, no glutamine	M36050	M36150
*MEM (Hanks' salts), 25 mM HEPES, L-glutamine	M29850	M38750
MEM (Hanks' salts), 25 mM HEPES, no glutamine	M38450	M38550
*MEM (Hanks' salts), 25 mM HEPES, non-essential amino acids, L-glutamine	M31250	M35650
MEM (Hanks' salts), 25 mM HEPES, non-essential amino acids, no glutamine	M32750	M32850
*MEM (Hanks' salts), L-glutamine	M29350	M38850
MEM (Hanks' salts), no glutamine	M31650	M38650
*MEM (Hanks' salts), non-essential amino acids, L-glutamine	M31150	M33750
MEM (Hanks' salts), non-essential amino acids, no glutamine	M32950	M33650
*RPMI 1640, 25 mM HEPES, L-glutamine	M30250	M30750
RPMI 1640, 25 mM HEPES, no glutamine	M30050	M30550
*RPMI 1640, L-glutamine	M30150	M30350
RPMI 1640, no glutamine	M30450	M30650

*Bio-Techne also offers these media with GlutaminePlus, an alternative to L-glutamine that can improve cell viability and growth.

R&D Systems™ Fetal Bovine Serum

Product	Catalog #s	
	Regular	Heat Inactivated
Fetal Bovine Serum - Optima	S12450	S12450H
Fetal Bovine Serum - Premium	S11150	S11150H
Fetal Bovine Serum - Premium Select	S11550	S11550H
Fetal Bovine Serum - Charcoal/Dextran Treated	S11650	S11650H
Fetal Bovine Serum - Dialyzed 12-14kD	S12850	S12850H
Fetal Bovine Serum - Embryonic Stem Cell Qualified	S10250	S10250H
Fetal Bovine Serum - Hybridoma Qualified	S10650	S10650H
Fetal Bovine Serum - TET Tested	S10350	S10350H

Serum is available in 50 mL, 100 mL, and 500 mL volumes.

R&D Systems™ Cell Culture Supplements

Product	Catalog #
N-2 Plus Media Supplement	AR003
N-2 MAX Media Supplement (100x)	AR009
N21-MAX Media Supplement (50x)	AR008
N21-MAX Insulin Free Media Supplement (50x)	AR010
N21-MAX Vitamin A Free Media Supplement (50x)	AR012
Human Holo-Transferrin	2914-HT

R&D Systems™ Cell Culture Kits

Product	Catalog #
NeuroXVivo™ Motor Neuron Culture Kit	CDK016

Cell Culture Products, continued

R&D Systems™ Extracellular Matrix Molecules

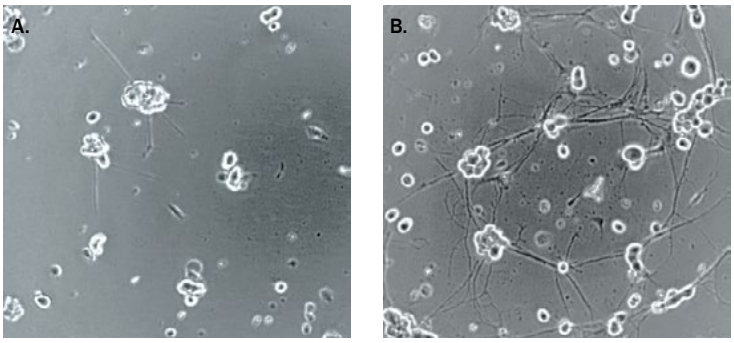
Molecule	Species	Catalog #	Source
Agrin	Human	6624-AG	CHO
Agrin (N-Terminal)	Human	8909-AG	CHO
Agrin	Rat	550-AG	*Sf* 21
Decorin	Human	143-DE	*Sf* 21
Decorin	Mouse	1060-DE	NS0
Fibronectin	Human	1918-FN	Plasma
Fibronectin	Human	4305-FN	NS0
Fibronectin (Cultrex®), PathClear®	Human	3420-001-01	Human
Fibronectin, ACFP	Human	ACFP4305B	*Sf* 9
Fibronectin, GMP	Human	4305B-GMP	*Sf* 9
Fibronectin, Fragment 2	Human	3225-FN	NS0
Fibronectin, Fragment 3	Human	3938-FN	NS0
Fibronectin, Fragment 4	Human	3624-FN	NS0
Fibronectin	Bovine	1030-FN	Plasma
Nidogen-1/Entactin	Human	2570-ND	NS0
F-Spondin/SPON1	Human	3135-SP	NS0
F-Spondin/SPON1	Mouse	7950-SP	NS0
R-Spondin 1	Human	4645-RS	CHO
R-Spondin 1	Mouse	3474-RS	*E. coli*
R-Spondin 1	Mouse	7150-RS	CHO
R-Spondin 2	Human	3266-RS	NS0
R-Spondin 2	Mouse	6946-RS	CHO
R-Spondin 3	Human	3500-RS	CHO
R-Spondin 3	Mouse	4120-RS	CHO
R-Spondin 4	Human	4575-RS	CHO
R-Spondin 4	Mouse	4106-RS	CHO
Tenascin C	Human	3358-TC	NS0
Tenascin R	Human	3865-TR	NS0
Tenascin XB2	Human	6999-TN	CHO
Testican 1/SPOCK1	Human	2327-PI	NS0
Testican 2/SPOCK2	Human	2328-PI	NS0
Vitronectin	Human	2349-VN	Plasma
Vitronectin	Human	2308-VN	NS0
Vitronectin (Cultrex), PathClear	Human	3421-001-01	Human
Vitronectin	Bovine	2348-VN	Plasma

Additional R&D Systems™ Cell Adhesion Molecules

Product	Catalog #
Cultrex® Poly-D-Lysine	3439-100-01
Cultrex Poly-L-Lysine	3438-100-01
Cultrex Poly-L-Ornithine Solution	3436-100-01

R&D Systems™ Basement Membrance Extracts (BME)

Product	Catalog #
Cultrex® Bovine Collagen I	3442-050-01
Cultrex Rat Collagen I	3440-100-01
Cultrex Rat Collagen I (Lower Viscosity)	3443-100-01
Cultrex 3-D Culture Matrix Rat Collagen I	3447-020-01
Cultrex Mouse Collagen IV	3410-010-02
Cultrex Mouse Laminin I, Pathclear®	3400-010-02
Cultrex Antibiotic-free Mouse Laminin I, Pathclear	3401-010-02
Cultrex 3-D Culture Matrix Laminin I	3446-005-01
Cultrex BME, Pathclear	3432-005-01
Cultrex Reduced Growth Factor BME, Pathclear	3433-005-01
Cultrex BME, Type 2, Pathclear	3532-005-02
Cultrex Reduced Growth Factor BME, Type 2, Pathclear	3533-005-02
Cultrex 3-D Culture Matrix Reduced Growth Factor BME, Pathclear	3445-001-01



F-Spondin-Induces Neurite Outgrowth. Embryonic chick dorsal root ganglion neurons (E13) were cultured in the absence (**A**) and presence (**B**) of Recombinant Human F-Spondin/SPON1 (R&D Systems, Catalog # 3135-SP) immobilized on a nitrocellulose-coated microplate. The presence of F-Spondin significantly enhanced neurite outgrowth.

Abbreviation Key: ACFP (Animal Component-Free Process), GMP (Good Manufacturing Practices),
Source Key: CHO (Chinese hamster ovary cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

R&D Systems™ and Tocris™ Antibiotics

Product	Brand	Catalog #
Ampicillin sodium salt	Tocris	5503
Antibiotic-Antimycotic (100X)	R&D Systems	B22110
Bafilomycin A1	Tocris	1334
Blasticidin S	Tocris	5502
Carbenicillin disodium salt	Tocris	5507
Clindamycin	Tocris	4822
Cycloheximide	Tocris	970
G418	Tocris	4131
Gentamicin (10 mg/mL)	R&D Systems	B20192
Ionomycin	Tocris	1704
Kanamycin	Tocris	5505
Leptomycin B	Tocris	1987
Oligomycin	Tocris	4110
Penicillin-Streptomycin 10/10 (100X)	R&D Systems	B21210
Penicillin-Streptomycin 5/5 (100X)	R&D Systems	B21110
Puromycin	Tocris	4089
Tunicamycin	Tocris	3516

R&D Systems™ and Tocris™ Substrates and Chelators

Product	Brand	Catalog #
BAPTA	Tocris	2786
BAPTA AM	Tocris	2787
Bovine Serum Albumin	Tocris	5217
Calcium chloride dihydrate	Tocris	3148
CHAPS	Tocris	3172
D-(+)-Glucose	Tocris	5504
DMSO, sterile filtered	Tocris	3176
EDTA	Tocris	2811
Glycerol	Tocris	5220
MTT	Tocris	5224
Ponceau S Staining Solution	Tocris	5225
Potassium chloride	Tocris	3147
Sodium bicarbonate	Tocris	3152
Sodium bicarbonate, powder	R&D Systems	R39150
Sodium citrate	Tocris	3161
TRIS base	Tocris	3163

R&D Systems™ and Tocris™ Buffers, Solutions and Additional Reagents

Product	Brand	Catalog #
ACSF	Tocris	3525
EBSS, Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B32750
EBSS, Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B31150
EBSS, no Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B31350
EBSS, no Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B31250
L-Glutamine (Ala-Gln)	Tocris	5823
L-Glutamine - 200 mM (100X)	R&D Systems	B90010
L-Glutamine, powder	R&D Systems	R90010
GlutaminePlus – 200 mM (100X)	R&D Systems	B90210
GlutaminePlus, powder	R&D Systems	R90210
HEPES	Tocris	3173
HEPES Sodium salt	Tocris	3174
HEPES (Ultra Pure), powder	R&D Systems	R35150
HEPES Buffer Solution (1 M)	R&D Systems	B35110
HBSS, Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B32450
HBSS, Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B32150
HBSS, no Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B32350
HBSS, no Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B32250
PBS, 100 Tablets	Tocris	5564
Dulbecco's PBS	Tocris	3156
DPBS, Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B30150
DPBS, Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B30750
DPBS, low glucose, sodium pyruvate, Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B30450
DPBS, low glucose, sodium pyruvate, Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B30050
DPBS, low glucose, sodium pyruvate, no Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B30650
DPBS, low glucose, sodium pyruvate, no Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B30550
DPBS, no Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B30250
DPBS, no Ca^{2+} and Mg^{2+} salts, phenol red	R&D Systems	B30850
Trypsin 2.5% (10X)	R&D Systems	B81710
Water, sterile filtered	Tocris	3179

R&D Systems™ Culture Microplates

Product	Catalog #
Clear Polystyrene Microplate	DY990
Human Fibronectin-Coated Microplate	CWP001

R&D Systems™ Mycoplasma Detection

Product	Catalog #
MycoProbe™ Mycoplasma Detection Kit	CUL001B

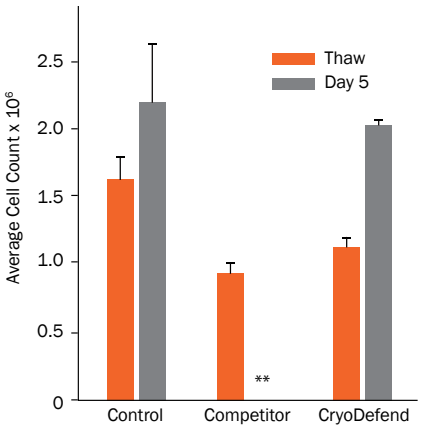
R&D Systems™ and Tocris™ Neural Stem Cell Products

Experts in Stem Cell Workflow

offering solutions to make experimental planning easier.

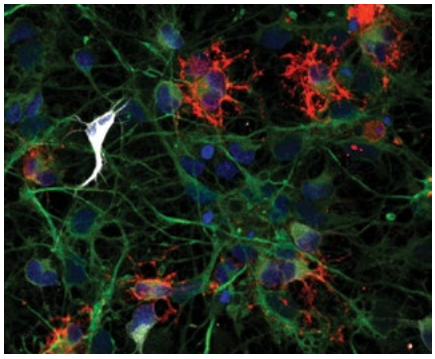
- **We Have You Covered.**
  Through isolation, differentiation, verification, and investigation, we have products to make your workflow efficient and reliable.
- **Minimize Experimental Variability.**
  Our kits quickly verify stem cell populations, increasing consistency across experiments. Reducing experimental variability saves time and allows for more confident data analysis.
- **Don't Wait to Differentiate.**
  All-in-one kits for differentiation of neural (NSC), embryonic, (ESC), and induced pluripotent (iPSC) stem cells. We'll help you make it look easy.

Culture and Expansion Products



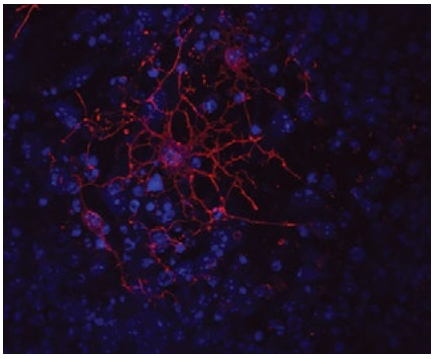
Superior Recovery of Rat Neural Progenitor Cells from CryoDefend™-Stem Cells Media Compared to Competitor Cryopreservation Media. Rat Cortical Stem Cells (Catalog # NSC001) were frozen (2 × 10^6 cells/vial) in control (10% BSA/10% DMSO), competitor, or CryoDefend-Stem Cells media (Catalog # CCM018). Cell viability was assessed at the time of thaw (orange bars) and after 5 days in culture (gray bars) in DMEM/F12 media supplemented with N-2 MAX (Catalog # AR009) and Recombinant Human FGF basic/FGF2/bFGF (145 aa) (Catalog # 4114-TC). All cited reagents are from R&D Systems.** indicates low recovery yield.

Identification and Verification Kits



β-III Tubulin, GFAP, and Oligodendrocyte Marker O4 in Differentiated Rat Cortical Stem Cells. Rat cortical stem cells (Catalog # NSC001) were differentiated into neurons, astrocytes, and oligodendrocytes using the Human/Mouse/Rat Neural Lineage Functional Identification Kit (Catalog # SC028). Differentiated cells were stained with cell-specific antibodies from the Human/Mouse/Rat Neural 3-Color Immunocytochemistry Kit (Catalog # SC024). Neurons were stained with a NL637-Conjugated Mouse Anti-Neuron-Specific β-III Tubulin Monoclonal Antibody (Clone TuJ-1; white), astrocytes were stained with a NL493-Conjugated Sheep Anti-GFAP Antigen Affinity-Purified Polyclonal Antibody (green), and oligodendrocytes were stained with a NL557-Conjugated Mouse Anti-Oligodendrocyte Marker O4 Monoclonal Antibody (red). The nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

Differentiation Kits



Mouse Oligodendrocytes Generated Using the Oligodendrocyte Differentiation Kit. D3 mouse embryonic stem cells were expanded in KO-ES media and differentiated into oligodendrocytes using the Mouse Oligodendrocyte Differentiation Kit (Catalog # SC004). An oligodendrocyte phenotype was detected using a Mouse Anti-Human/Mouse/Rat/Chicken Oligodendrocyte Marker O4 Monoclonal Antibody (Catalog # MAB1326) followed by staining with the NL557-Conjugated Goat Anti-Mouse IgM Secondary Antibody (Catalog # NL019; red). The nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

R&D Systems™ Cortical Stem Cells and Cryopreservation Medium

Product	Catalog #	Description
Rat Primary Cortical Stem Cells	NSC001	Isolated from E14.5 Sprague-Dawley rats; 3 × 10^6 cells/vial
Mouse Primary Cortical Stem Cells	NSC002	Isolated from E14.5 CD-1 mice; 2 × 10^6 cells/vial
CryoDefend™-Stem Cells	CCM018	Media for protein-free cryopreservation of stem cells

R&D Systems™ Neural Stem Cell Kits

Product	Catalog #	Description	Species
Neural Lineage Functional Identification Kit	SC028	Verify multipotency of human, mouse, and rat neural progenitor cells (NPCs) by functional differentiation	Human, Mouse, Rat
Neural 3-Color Immunocytochemistry Kit	SC024	Characterize human, mouse, and rat neural cells differentiated from NPCs	Human, Mouse, Rat
Neural Progenitor Cell Marker Antibody Panel	SC025	Contains a collection of neural progenitor markers to verify multipotency	Human, Mouse, Rat
Dopaminergic Neuron Differentiation Kit	SC001B	Differentiates human and mouse ESC/iPSC cells into dopaminergic neurons under serum-free conditions	Human, Mouse
Oligodendrocyte Differentiation Kit	SC004	Differentiates mouse ES/iPS cells into oligodendrocytes under serum-free conditions	Mouse
StemXVivo™ Neural Progenitor	SC035	Differentiate human pluripotent stem cells into multipoint NPCs	Human

R&D Systems™ Proteins for Neural Stem Cell Research

Molecule	Species	Catalog #	Source
BDNF	Human	11166-BD	CHO
BMP-2	Human, Mouse, Rat	355-BM	CHO
BMP-2, GMP	Human	355-GMP	CHO
BMP-4	Human	314-BP	NS0
BMP-4, GMP	Human	314-GMP	NS0
BMP-4	Mouse	5020-BP	CHO
CNTF	Human	257-NT	*E. coli*
CNTF	Rat	557-NT	*E. coli*
EGF	Human	236-EG	*E. coli*
EGF, Animal Free	Human	AFL236	*E. coli*
EGF, GMP	Human	236-GMP	*E. coli*
EGF	Mouse	2028-EG	*E. coli*
EGF, Animal Free	Mouse	AFL2028	*E. coli*
EGF	Rat	3214-EG	*E. coli*
FGF basic/FGF2/bFGF	Human	3718-FB	*E. coli*
FGF basic/FGF2/bFGF (145 aa), GMP	Human	3718-GMP	*E. coli*
FGF basic/FGF2/bFGF	Mouse	3139-FB	*E. coli*
FGF basic/FGF2/bFGF	Rat	3339-FB	*E. coli*
FGF-8b	Human, Mouse	423-F8	*E. coli*
FGF-10	Human	345-FG	*E. coli*
FGF-10	Mouse	6224-FG	*E. coli*
FGF-10	Rat	7804-FG	*E. coli*
GDNF	Human	212-GD	NS0
GDNF, GMP	Human	212-GMP	NS0
GDNF	Rat	512-GF	*Sf* 21
IGF-I/IGF-1	Human	291-G1	*E. coli*
IGF-I/IGF-1, Animal Free	Human	AFL291	*E. coli*
IGF-I/IGF-1, GMP	Human	291-GMP	*E. coli*

Molecule	Species	Catalog #	Source
IGF-I/IGF-1	Mouse	791-MG	*E. coli*
IGF-I/IGF-1	Rat	4326-RG	*E. coli*
β-NGF	Human	256-GF	NS0
β-NGF	Mouse	1156-NG	NS0
β-NGF	Rat	556-NG	*Sf* 21
NT-3	Human	267-N3	*Sf* 21
NT-4	Human	268-N4	*Sf* 21
NT-4	Mouse	3236-N4	NS0
PDGF	Human	120-HD	Human Platelets
PDGF-AA	Human	221-AA	*E. coli*
PDGF-AA, Animal Free	Human	AFL221	*E. coli*
PDGF-AA, GMP	Human	221-GMP	*E. coli*
PDGF-AA	Rat	1055-AA	*E. coli*
PDGF-AB	Human	222-AB	*E. coli*
PDGF-AB	Rat	1115-AB	*E. coli*
PDGF-BB	Human	220-BB	*E. coli*
PDGF-BB, GMP	Human	220-GMP	*E. coli*
PDGF-BB	Rat	520-BB	*E. coli*
Sonic Hedgehog/Shh (High Activity)	Human	8908-SH	HEK293
Sonic Hedgehog /Shh (C24II, N-Terminus)	Human	1845-SH	*E. coli*
Sonic Hedgehog /Shh (C24II, N-Terminus), GMP	Human	1845-GMP	*E. coli*
Sonic Hedgehog /Shh (C25II, N-Terminus)	Mouse	464-SH	*E. coli*
VEGF 164	Mouse	493-MV	*Sf* 21
VEGF 164	Rat	564-RV	NS0
VEGF 165	Human	293-VE	*Sf* 21
VEGF, GMP	Human	293-GMP	*Sf* 9

Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

Neural Stem Cell Products, continued

Tocris™ Small Molecules for Stem Cell Workflows and Neuronal Differentiation

Product	Catalog #	Description
16,16-Dimethyl Prostaglandin E2	4027	Synthetic prostaglandin E2 (Tocris, Catalog # 2296) derivative; regulates hematopoietic stem cell development
A 83-01	2939	Selective inhibitor of TGF-β RI, ALK4 and ALK7; maintains self-renewal of human iPSCs
CHIR 99021	4423	Highly selective GSK-3 inhibitor; enables reprogramming of mouse embryonic fibroblasts into iPSCs
Compound E	6476	γ-Secretase inhibitor; induces neuronal differentiation
Cyclopamine	1623	Inhibitor of Hedgehog (Hh) signaling; depletes stem-like cancer cells in glioblastoma
DAPT	2634	γ-Secretase inhibitor; induces neuronal differentiation
Dibutyryl-cAMP, sodium salt	1141	Cell-permeable cAMP analog; promotes differentiation of human PSCs to dopaminergic neurons
DMH-1	4126	BMP receptor ALK2 inhibitor; promotes iPSC neurogenesis in combination with SB 431542 (Tocris, Catalog # 1614)
Fluoxetine	0927	5-HT reuptake inhibitor; induces differentiation of neuronal precursors
Forskolin	1099	Adenylyl Cyclase activator; induces neuronal differentiation
ISX 9	4439	Neurogenic agent; induces neuronal differentiation of SVZ progenitors
LDN 193189	6053	Potent and selective ALK2 and ALK3 inhibitor; promotes neural induction of human PSCs
Metformin	2864	Activator of LKB1/AMPK; enhances neurogenesis
ML 184	6668	Selective GPR55 agonist; promotes NSC proliferation and differentiation
P7C3	4076	Neuroprotective compound; enhances neurogenesis *in vivo*
PD 0325901	4192	Selective inhibitor of MEK1/2; enhances generation of iPSCs
Retinoic acid	0695	Endogenous Retinoic Acid Receptor agonist; promotes ESC differentiation in neurons; component of brain organoid differentiation media
SAG dihydrochloride	6390	Smo Receptor agonist; enhances neuronal differentiation of iPSCs into dopaminergic neurons
SB 431542	1614	Potent, selective inhibitor of TGF-β RI, ALK4 and ALK7; induces reprogramming of fibroblasts into iPSCs
SU 5402	3300	Potent FGFR and VEGFR inhibitor; attenuates Integrin β4-induced differentiation of NSCs
TCS 2210	3877	Inducer of neuronal differentiation in mesenchymal stem cells
Trazodone	6336	5-HT_{2A} and α_1 adrenoceptor antagonist; enhances differentiation of NPCs
TWS 119	3835	GSK-3β inhibitor; induces neuronal differentiation in ESCs
Valproic acid, sodium salt	2815	Histone Deacetylase inhibitor; enables induction of PSCs from somatic cells
Y-27632	1254	Selective ROCK inhibitor; increases survival rate of human ESCs undergoing cryopreservation

Tocris™ GMP Stem Cell Compounds

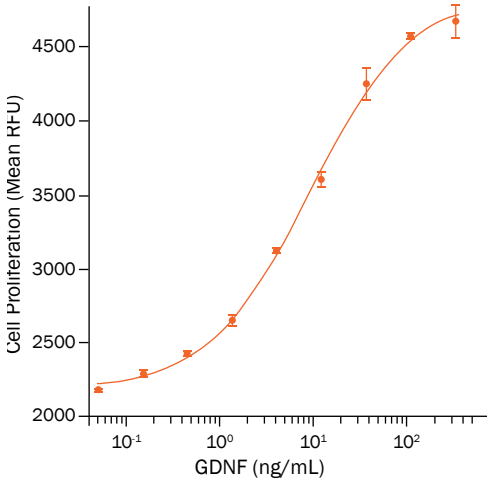
Product	Catalog #	Description
CHIR 99021	TB4423-GMP	CHIR 99021 synthesized to cGMP guidelines
DAPT	TB2634-GMP	DAPT synthesized to cGMP guidelines
SB 431542	TB1614-GMP	SB 431542 synthesized to cGMP guidelines
Y-27632	TB1254-GMP	Y-27632 synthesized to cGMP guidelines

R&D Systems™ Proteins

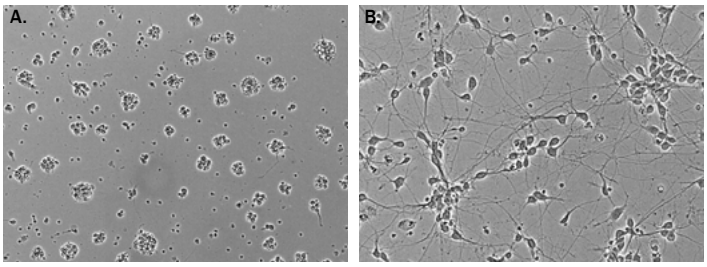
The Most Reputable Manufacturer

of biologically active recombinant and natural proteins for support of cultured cells.

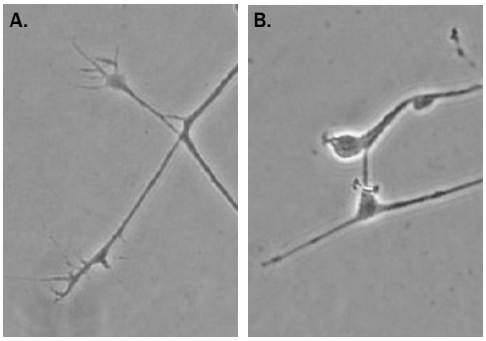
- **Guaranteed Performance, Always.**
  We back up our performance specifications with relevant bioassay data and every lot is tested before release to ensure consistent performance.
- **Custom Development and GMP Manufacturing.**
  We can make proteins to meet your specifications, including manufacturing using GMP guidelines for cell therapy.
- **Manufactured by Scientists, for Scientists.**
  Our team of over 100 protein scientists brings the most experience and know-how in the industry to the development and production of our proteins.



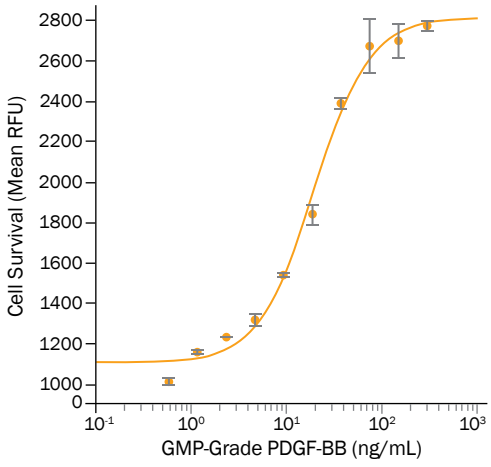
GDNF Induces Proliferation of Human Neuroblastoma Cells. The SH-SY5Y human neuroblastoma cell line was treated with increasing concentrations of Recombinant Human GDNF (Catalog # 212-GD). Cell proliferation was assessed using Resazurin (Catalog # AR002). All cited reagents are from R&D Systems.



Slit3 Stimulates Neurite Outgrowth. Embryonic rat cortical neurons (E16-E18) were cultured in the absence (**A**) or presence (**B**) of Recombinant Human Slit3 (aa 1120–1523, R&D Systems, Catalog # 9067-SL). The presence of the Slit3 protein significantly enhanced neurite outgrowth.



Semaphorin 3A-Induced Growth Cone Collapse. A fully extended chick dorsal root ganglion growth cone, grown in the presence of Recombinant Human β-NGF (Catalog # 256-GF), was untreated (**A**) or treated (**B**) with Recombinant Human Semaphorin 3A (Catalog # 1250-S3). Treatment with Semaphorin 3A induced growth cone collapse. All cited reagents are from R&D Systems.



Bioactivity of GMP-Grade Recombinant Human PDGF-BB. GMP-Grade Recombinant Human PDGF-BB (Catalog # 220-GMP) enhances survival of rat embryonic cortical neurons (E16–E18). Cell survival was assessed using Resazurin (Catalog # AR002). All cited reagents are from R&D Systems.

R&D Systems™ Neurotrophic and Neuronal Differentiation Factors

Select Activins

Molecule	Species	Catalog #	Source
Activin A	Human, Mouse, Rat	338-AC	CHO
Activin A, Animal Free™	Human, Mouse, Rat	AFL338	*E. coli*
Activin A, ACFP	Human, Mouse, Rat	ACFP338	*Sf* 9
Activin A, GMP	Human, Mouse, Rat	338-GMP	CHO
Latent Activin A	Human	9129-LA	CHO
Activin AB	Human	1066-AB	CHO
Activin AC	Human	4879-AC	CHO

Molecule	Species	Catalog #	Source
Activin B	Human	659-AB	CHO
Activin B	Mouse	8260-AB	HEK293
Activin C	Human	1629-AC	HEK293
Activin C	Mouse	489-AC	CHO
Inhibin A	Human	8506-AB	CHO
Inhibin A	Mouse	8346-IN	CHO

Select BMPs (Bone Morphogenetic Proteins)

Molecule	Species	Catalog #	Source
BMP-2	Human, Mouse, Rat	355-BEC	*E. coli*
BMP-2	Human, Mouse, Rat	355-BM	CHO
BMP-2, GMP	Human	355-GMP	CHO
BMP-2/BMP-6 Heterodimer	Human	7145-BP	*E. coli*
BMP-2/BMP-7 Heterodimer	Human	3229-BM	*E. coli*
BMP-3	Human	113-BP/CF	*E. coli*
BMP-3b/GDF-10	Human	1543-BP	*E. coli*
BMP-4	Human	314-BP	NS0
BMP-4, GMP	Human	314-GMP	NS0
BMP-4	Mouse	5020-BP	CHO
BMP-4/BMP-7 Heterodimer	Human	3727-BP	*E. coli*
BMP-5	Human	615-BMC	CHO

Molecule	Species	Catalog #	Source
BMP-5	Mouse	6176-BM	CHO
BMP-6	Human	507-BP	NS0
BMP-6	Mouse	6325-BM	CHO
BMP-7	Human	354-BP	CHO
BMP-7, GMP	Human	354-GMP	CHO
BMP-7	Mouse	5666-BP	CHO
BMP-9 (Latent)	Human	9624-BP	CHO
BMP-9	Human	3209-BP	CHO
BMP-9	Mouse	5566-BP	CHO
BMP-15/GDF-9B	Human	5096-BM	CHO

Select EGF Ligands

Molecule	Species	Catalog #	Source
Amphiregulin	Human	262-AR	*E. coli*
Amphiregulin	Mouse	989-AR	*E. coli*
Betacellulin/BTC	Human	261-CE	*E. coli*
Betacellulin/BTC	Mouse	1025-CE	*E. coli*
EGF	Human	236-EG	*E. coli*
EGF, Animal Free™	Human	AFL236	*E. coli*
EGF, GMP	Human	236-GMP	*E. coli*
EGF	Mouse	2028-EG	*E. coli*
EGF, Animal Free	Mouse	AFL2028	*E. coli*
EGF	Rat	3214-EG	*E. coli*
Pro-EGF (aa 21–1023)	Human	4289-EG	NS0
Pro-EGF (aa 29–1029)	Mouse	4095-EG	NS0
EGF-L6	Human	8638-EG	HEK293
EGF-L6	Mouse	4329-EG	NS0
Epigen	Human	6629-EP	*E. coli*
Epigen	Mouse	1127-EP	*E. coli*

Molecule	Species	Catalog #	Source
Epiregulin	Human	1195-EP	*E. coli*
Epiregulin	Mouse	1068-EP	*E. coli*
HB-EGF	Human	259-HE	*Sf* 21
LRIG1	Human	8504-LR	HEK293
LRIG1	Mouse	3688-LR	NS0
LRIG2	Human	1941-LR	CHO
LRIG3	Human	3495-LR	NS0
Neuregulin-1/NRG1	Human	5898-NR	NS0
Neuregulin-1/NRG1	Mouse	9875-NR	NS0
NRG1 (Isoform SMDF)	Human	378-SM	*Sf* 21
NRG1-α/HRG1-α (EGF Domain)	Human	296-HR	*E. coli*
NRG1-β1/HRG1-β1 (ECD)	Human	377-HB	*E. coli*
NRG1-β1/HRG1-β1 (EGF Domain)	Human	396-HB	*E. coli*
NRG1-β1/HRG1-β1, GMP	Human	396-GMP	*E. coli*
TGF-α	Human	239-A	*E. coli*

Abbreviation Key: ACFP (Animal Component-Free Process), ECD (Extracellular Domain), GMP (Good Manufacturing Practices), TC (Tissue Culture)
Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*), *T. ni* (*Trichoplusia ni*)

Select FGF Ligands

Molecule	Species	Catalog #	Source
FGF acidic/FGF1	Mouse	4686-FA	*E. coli*
FGF acidic/FGF1	Bovine	132-FA	Bovine Brain
FGF acidic/FGF1, Animal-Free	Human	AFL232	*E. coli*
FGF acidic/FGF1 (aa 2–155)	Human	231-BC	*E. coli*
FGF acidic/FGF1 (aa 16–155)	Human	232-FA	*E. coli*
FGF basic/FGF2/bFGF	Mouse	3139-FB	*E. coli*
FGF basic/FGF2/bFGF	Rat	3339-FB	*E. coli*
FGF basic/FGF2/bFGF	Bovine	133-FB	Bovine Brain
FGF basic/FGF2/bFGF	Bovine	2099-FB	*E. coli*
FGF basic/FGF2/bFGF, Animal-Free	Bovine	AFL2099	*E. coli*
FGF basic/FGF2/bFGF (145 aa)	Human	3718-FB	*E. coli*
FGF basic/FGF2/bFGF (145 aa, TC Grade)	Human	4114-TC	*E. coli*
FGF basic/FGF2/bFGF (145 aa), GMP	Human	3718-GMP	*E. coli*
FGF basic/FGF2/bFGF (157 aa)	Human	234-FSE	*E. coli*
FGF-3	Human	1206-F3	*E. coli*
FGF-4	Human	235-F4	*E. coli*
FGF-4, Animal-Free	Human	AFL235	*E. coli*
FGF-4	Mouse	5846-F4	*E. coli*
FGF-4 (aa 67–202)	Mouse	7486-F4	*E. coli*
FGF-4 (aa 71–206)	Human	7460-F4	*E. coli*
FGF-5	Human	237-F5	*E. coli*
FGF-6	Human	238-F6	*E. coli*
FGF-6	Mouse	5750-F6	*E. coli*
FGF-6 (aa 67–208)	Human	6829-F6	*E. coli*
KGF/FGF-7	Human	251-KG	*E. coli*

Molecule	Species	Catalog #	Source
KGF/FGF-7, GMP	Human	251-GMP	*E. coli*
KGF/FGF-7	Mouse	5028-KG	*E. coli*
FGF-8a	Human	4745-F8	*E. coli*
FGF-8b	Human, Mouse	423-F8	*E. coli*
FGF-8c	Mouse	424-FC	*E. coli*
FGF-8e	Human	4746-F8	*E. coli*
FGF-8f	Human	5027-FF	*E. coli*
FGF-9	Human	273-F9	*Sf* 21
FGF-9	Mouse	7399-F9	*E. coli*
FGF-10	Human	345-FG	*E. coli*
FGF-10	Mouse	6224-FG	*E. coli*
FGF-10	Rat	7804-FG	*E. coli*
FGF-12	Human	2246-FG	*E. coli*
FGF-16	Human	1212-FG	*E. coli*
FGF-17	Human	319-FG	*E. coli*
FGF-17	Mouse	7400-FG	*E. coli*
FGF-18	Human	8988-F18	*E. coli*
FGF-19	Human	969-FG	*E. coli*
FGF-20	Human	2547-FG	*E. coli*
FGF-21	Human	2539-FG	*E. coli*
FGF-21	Mouse	8409-FG	NS0
FGF-22	Human	3867-FG	*E. coli*
FGF-23	Human	2604-FG	NS0
FGF-23	Mouse	2629-FG	NS0

Select GDNF Family Ligands

Molecule	Species	Catalog #	Source
Artemin	Human	2589-AR	*E. coli*
Artemin	Mouse	1085-AR	*E. coli*
GDNF	Human	212-GD	NS0
GDNF, GMP	Human	212-GMP	NS0
GDNF	Rat	512-GF	*Sf* 21

Molecule	Species	Catalog #	Source
Neurturin	Human	1297-NE	*E. coli*
Neurturin	Mouse	477-MN	*E. coli*
Persephin	Human	2388-PS	*E. coli*
Persephin	Mouse	2479-PS	*E. coli*

Select Hedgehogs

Molecule	Species	Catalog #	Source
Desert Hedgehog (N-Terminus)	Human	4777-DH	*E. coli*
Desert Hedgehog (C23II, N-Terminus)	Mouse	733-DH	*E. coli*
Indian Hedgehog (C28II, N-Terminus)	Human, Mouse	1705-HH	*E. coli*
Sonic Hedgehog/Shh (N-Terminus)	Human	1314-SH	*E. coli*
Sonic Hedgehog/Shh (High Activity)	Human	8908-SH	HEK293
Sonic Hedgehog/Shh (N-Terminus), GMP	Human	1314-GMP	*E. coli*
Sonic Hedgehog/Shh (N-Terminus)	Mouse	461-SH	*E. coli*

Molecule	Species	Catalog #	Source
Sonic Hedgehog/Shh (C24II, N-Terminus)	Human	1845-SH	*E. coli*
Sonic Hedgehog/Shh (C24II, N-Terminus), GMP	Human	1845-GMP	*E. coli*
Sonic Hedgehog/Shh (C25II, N-Terminus)	Mouse	464-SH	*E. coli*

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Neurotrophic and Neuronal Differentiation Factors, continued

Select IGF Ligands

Molecule	Species	Catalog #	Source
IGF-I/IGF-1	Human	291-G1	*E. coli*
IGF-I/IGF-1, Animal-Free	Human	AFL291	*E. coli*
IGF-I/IGF-1, GMP	Human	291-GMP	*E. coli*
IGF-I/IGF-1	Mouse	791-MG	*E. coli*
IGF-I/IGF-1	Rat	4326-RG	*E. coli*

Molecule	Species	Catalog #	Source
LR3 IGF-I/IGF-1	Human	8335-G1	*E. coli*
LR3 IGF-I/IGF-1, GMP	Human	8335D-GMP	*E. coli*
IGF-II/IGF-2	Human	292-G2	*E. coli*
IGF-II/IGF-2	Mouse	792-MG	*E. coli*

Select Neurotrophin/Trk Family Ligands

Molecule	Species	Catalog #	Source
BDNF	Human	11166-BD	CHO
β-NGF	Human	256-GF	NS0
β-NGF	Mouse	1156-NG	NS0
β-NGF	Rat	556-NG	*Sf* 21
β-NGF	Rat	7815-NG	CHO

Molecule	Species	Catalog #	Source
NT-3	Human	267-N3	*Sf* 21
NT-3, GMP	Human	267-GMP	*Sf* 21
NT-4	Human	268-N4	*Sf* 21
NT-4	Mouse	3236-N4	NS0

Select PDGF Family Ligands

Molecule	Species	Catalog #	Source
PDGF	Human	120-HD	Human Platelets
PDGF-AA	Human	221-AA	*E. coli*
PDGF-AA, Animal Free	Human	AFL221	*E. coli*
PDGF-AA, GMP	Human	221-GMP	*E. coli*
PDGF-AA	Rat	1055-AA	*E. coli*
PDGF-AB	Human	222-AB	*E. coli*
PDGF-AB	Rat	1115-AB	*E. coli*
PDGF-BB	Human	220-BB	*E. coli*

Molecule	Species	Catalog #	Source
PDGF-BB, Animal-Free	Human	AFL220	*E. coli*
PDGF-BB, GMP	Human	220-GMP	*E. coli*
PDGF-BB	Rat	520-BB	*E. coli*
PDGF-CC	Human	1687-CC	*E. coli*
PDGF-CC	Mouse	1447-PC	*E. coli*
PDGF-DD	Human	1159-SB	NS0
PDGF-D	Mouse	9738-SB	NS0

Select TGF-β Family Ligands

Molecule	Species	Catalog #	Source
LAP (TGF-β1)	Human	246-LP	*Sf* 21
Latent TGF-β1	Human	299-LT	CHO
TGF-β1	Human	100-B	Human Platelets
TGF-β1	Human	7754-BH	HEK293
TGF-β1, ACFP	Human	ACFP240	*Sf* 9
TGF-β1, GMP	Human	240-GMP	CHO
TGF-β1, GMP, ACFP	Human	240AF-GMP	*Sf* 9
TGF-β1	Mouse	7666-MB	CHO

Molecule	Species	Catalog #	Source
TGF-β1.2	Human	304-B3	*Sf* 21
TGF-β2	Human	302-B2	NS0
TGF-β2, ACFP	Human	ACFP302	*Sf* 9
TGF-β2	Mouse	7346-B2	CHO
TGF-β3	Human	243-B3	*Sf* 21
TGF-β3	Human	8420-B3	CHO
TGF-β3, GMP	Human	243-GMP	*Sf* 9

Abbreviation Key: ACFP (Animal Component-Free Process), ECD (Extracellular Domain), GMP (Good Manufacturing Practices), TC (Tissue Culture)
Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*), *T. ni* (*Trichoplusia ni*)

Select VEGF Family Ligands

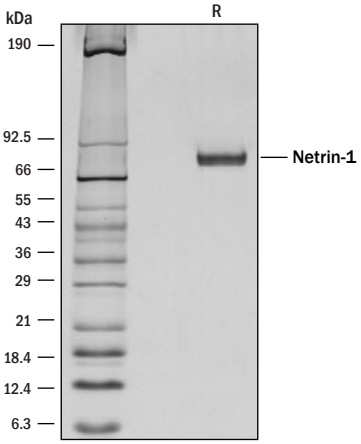
Molecule	Species	Catalog #	Source
PlGF	Human	264-PGB	*E. coli*
PlGF-2	Human	6837-PL	CHO
PlGF-2	Mouse	465-PL	*Sf* 21
PlGF-3	Human	7758-PL	*Sf* 21
PlGF-4	Human	8987-PL	HEK293
VEGF/PlGF Heterodimer	Human	297-VP	*E. coli*
VEGF, ACFP	Human	ACFP293	*Sf* 9
VEGF (aa 207–318)	Human	298-VS	*E. coli*
VEGF 111	Human	5336-VE	*E. coli*
VEGF 111b	Human	10036-VE	*E. coli*
VEGF 120	Mouse	494-VE	*E. coli*
VEGF 121 (aa 207–327)	Human	4644-VS	*E. coli*
VEGF 145 (aa 27–171)	Human	7626-VE	*E. coli*
VEGF 162	Human	2347-VE	NS0
VEGF 164	Mouse	493-MV	*Sf* 21
VEGF 164	Rat	564-RV	NS0

Molecule	Species	Catalog #	Source
VEGF 164	Bovine	8917-BV	CHO
VEGF 165	Human	293-VE	*Sf* 21
VEGF 165 (Extended Isoform)	Human	9018-VE	CHO
VEGF 165, GMP	Human	293-GMP	*Sf* 9
VEGF 165b	Human	3045-VE	*Sf* 21
VEGF 188	Mouse	7916-MV	HEK293
VEGF 189 (aa 27–215)	Human	8147-VE	CHO
VEGF-B 167	Human	751-VE	*E. coli*
VEGF-B 167	Mouse	2595-VE	*E. coli*
VEGF-B 186	Mouse	767-VE	*Sf* 21
VEGF-C	Human	9199-VC	CHO
VEGF-C (Cys156Ser)	Human	752-VC	NS0
VEGF-D	Human	622-VD	*Sf* 21
VEGF-D	Mouse	469-VD	*Sf* 21

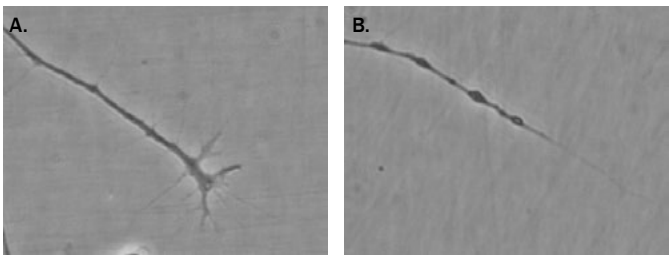
Select Wnt Ligands

Molecule	Species	Catalog #	Source
Wnt-1/sFRP-1 Complex	Mouse	9735-WN	CHO
Wnt-3a	Human	5036-WN	CHO
Wnt-3a (High Purity)	Human	5036-WNP	CHO
Wnt-3a, GMP	Human	5036-GMP	CHO
Wnt-3a	Mouse	1324-WN	CHO
Wnt-3a (High Purity)	Mouse	1324-WNP	CHO
Wnt-4	Human	6076-WN	CHO

Molecule	Species	Catalog #	Source
Wnt-4	Mouse	475-WN	CHO
Wnt-5a	Human, Mouse	645-WN	CHO
Wnt-5b	Human	7347-WN	CHO
Wnt-5b	Mouse	3006-WN	CHO
Wnt-7a	Human	3008-WN	CHO
Wnt-11	Human	6179-WN	CHO



R&D Systems™ Netrin-1 Protein is Highly Pure. To highlight purity, Recombinant Mouse Netrin-1 (R&D Systems, Catalog # 1109-N1) was visualized on a silver stained 4–20% SDS polyacrylamide gel under reducing (**R**) conditions. A single band is observed at approximately 83 kDa (as indicated).



Semaphorin 6B-Induced Growth Cone Collapse. A fully extended chick dorsal root ganglion growth cone, grown in the presence of Recombinant Human β-NGF (Catalog # 256-GF), was untreated (**A**) or treated (**B**) with Recombinant Human Semaphorin 6B (Catalog # 2094-S6). Treatment with Semaphorin 6B induced growth cone collapse. All cited reagents are from R&D Systems.

R&D Systems™ Axon Guidance Cues

Select Netrins

Molecule	Species	Catalog #	Source
Netrin-1	Human	6419-N1	NS0
Netrin-1	Mouse	1109-N1	NS0
Netrin-1	Chicken	128-N1	NS0
Netrin-2	Chicken	127-N2	NS0
Netrin-4	Human	1254-N4	NS0

Molecule	Species	Catalog #	Source
Netrin-4	Mouse	1132-N4	NS0
Netrin-G1a	Mouse	1166-NG	*Sf* 21
Netrin-G2a	Mouse	2744-NG	NS0
NGL-1/LRRC4C	Human	4899-NR	NS0

Select Semaphorins

Molecule	Species	Catalog #	Source
Semaphorin 3A	Human	1250-S3	NS0
Semaphorin 3A	Mouse	5926-S3	CHO
Semaphorin 3B	Human	9518-S3	*T. ni*
Semaphorin 3B	Mouse	5440-S3	NS0
Semaphorin 3C	Human	5570-S3	NS0
Semaphorin 3C (Truncated)	Mouse	1728-S3	*Sf* 21
Semaphorin 3D	Human	9674-S3	CHO
Semaphorin 3D	Mouse	9386-S3	CHO
Semaphorin 3E	Human	3239-S3B	NS0
Semaphorin 3E	Mouse	3238-S3	*Sf* 21
Semaphorin 3F	Human	9878-S3	NS0
Semaphorin 3F (Truncated)	Mouse	3237-S3	NS0
Semaphorin 4A	Human	4694-S4	NS0
Semaphorin 4C	Human	6125-S4	NS0
Semaphorin 4C	Mouse	8394-S4	NS0
Semaphorin 4D/CD100	Human	7470-S4	CHO
Semaphorin 4D/CD100	Mouse	5235-S4	NS0
Semaphorin 4F	Human	10113-S4	CHO

Molecule	Species	Catalog #	Source
Semaphorin 4F	Mouse	7200-S4	NS0
Semaphorin 4G	Human	5840-S4	NS0
Semaphorin 4G	Mouse	6504-S4	NS0
Semaphorin 5A	Human	5896-S5	NS0
Semaphorin 5A	Mouse	6584-S5	CHO
Semaphorin 5B	Human	6680-S5	CHO
Semaphorin 5B	Mouse	6766-S5	CHO
Semaphorin 6A	Human	1146-S6	NS0
Semaphorin 6A	Mouse	9017-S6	*Sf* 21
Semaphorin 6B	Human	2094-S6	NS0
Semaphorin 6B	Mouse	2264-S6	HEK293
Semaphorin 6C	Human	2219-S6	NS0
Semaphorin 6C	Mouse	2108-S6	NS0
Semaphorin 6D	Human	2095-S6	NS0
Semaphorin 6D	Mouse	7067-S6	NS0
Semaphorin 7A	Human	2068-S7	NS0
Semaphorin 7A	Mouse	1835-S3	NS0

Select Slit Ligands

Molecule	Species	Catalog #	Source
Slit1	Human	6514-SL	CHO
Slit1 (aa 1129–1534)	Human	9848-SL	HEK293
Slit1	Mouse	5199-SL	CHO
Slit1 (aa 1126–1531)	Mouse	9879-SL	NS0
Slit2 (aa 26–1118)	Human	8616-SL	HEK293
Slit2 (aa 1122–1529)	Human	9379-SL	HEK293

Molecule	Species	Catalog #	Source
Slit2 (aa 26–900)	Mouse	5444-SL	CHO
Slit2 (aa 1114–1521)	Mouse	9430-SL	NS0
Slit3 (aa 34–1116)	Human	9255-SL	HEK293
Slit3 (aa 1120–1523)	Human	9067-SL	HEK293
Slit3 (aa 34–1116)	Mouse	9296-SL	NS0
Slit3 (aa 1117–1523)	Mouse	9295-SL	NS0

Select SLITRKs

Molecule	Species	Catalog #	Source
SLITRK1	Human	3009-SK	NS0
SLITRK1	Mouse	9460-SK	NS0
SLITRK2	Human	8947-SK	NS0
SLITRK2	Mouse	9965-SK	NS0
SLITRK3	Human	8957-SK	NS0

Molecule	Species	Catalog #	Source
SLITRK4	Human	8945-SK	NS0
SLITRK5	Human	2587-SK	NS0
SLITRK5	Mouse	9520-SK	NS0
SLITRK6	Human	8985-SK	NS0
SLITRK6	Mouse	1812-SK	NS0

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Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*), *T. ni* (*Trichoplusia ni*)

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  Many probes we offer are exclusive to us.
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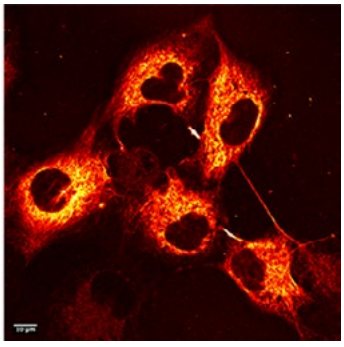
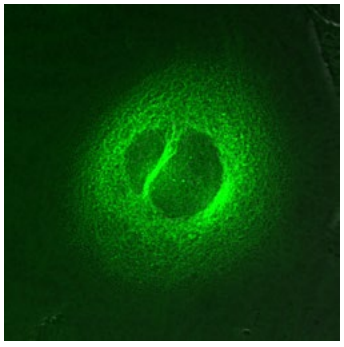
Tocris™ Fluorescent Reagents

Product	Catalog #	Description
CRANAD 2	4803	Near-infrared probe that detects Aβ40 aggregates
DAPI	5748	Fluorescent DNA stain
D-Luciferin sodium salt	5427	Firefly luciferase substrate; cell permeable.
FFN 102	5200	Selective fluorescent substrate of DAT and VMAT2
FFN 200	5911	Selective fluorescent VMAT2 substrate
FFN 270	6717	Fluorescent substrate for NET and VMAT2
Flutax 1	2226	Fluorescent taxol derivative; binds microtubules
Flutax 2	6254	Green fluorescent taxol derivative; binds microtubules
Hoechst 33342	5117	Fluorescent dye for labeling DNA
K 114	3144	Amyloid fibril-specific fluorescent dye
L 012 sodium salt	5085	Chemiluminescent ROS and RNS indicator
Methoxy-X04	4920	Fluorescent Aβ detector; brain penetrant
MitoMark Green I	6444	Green fluorescent mitochondrial stain; cell permeable
MitoMark Red I	6445	Red fluorescent mitochondrial stain; cell permeable
Phalloidin-FITC	5782	Green fluorescent cytoskeleton stain
ReZolve-ER™	6640	Fluorescent ER and plasma membrane stain
Sulforhodamine 101	5146	Red fluorescent dye; selective astrocyte marker
Taxol Janelia Fluor® 646	6266	Red fluorescent taxol derivative; binds microtubules



Visualization of Microtubules in COS-7 Cells. Taxol Janelia Fluor® 646 (3 µM; Tocris, Catalog # 6266) was used to visualize the microtubule cytoskeleton (red) in COS-7 African green monkey kidney fibroblast-like cells. This probe only fluoresces upon binding to the cytoskeleton, enabling its use in no-wash protocols. *Image kindly provided by Prof. Christian Soeller, University of Exeter; acquired by Evelina Lucinskaite, Anna Meletiou and Alexander Clowsley.*



Visualization of Microtubules in Live HeLa Cells with Flutax 1. Flutax 1 (2 µM; Tocris, Catalog # 2226) was used to visualize microtubules (green) in live HeLa human cervical epithelial carcinoma cells. Flutax 1 staining can only be done in live cells as staining is not retained after fixation (photobleaching).

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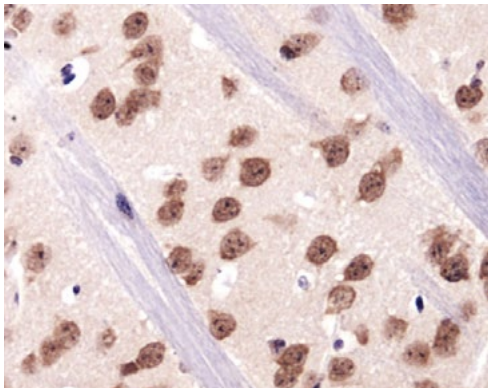
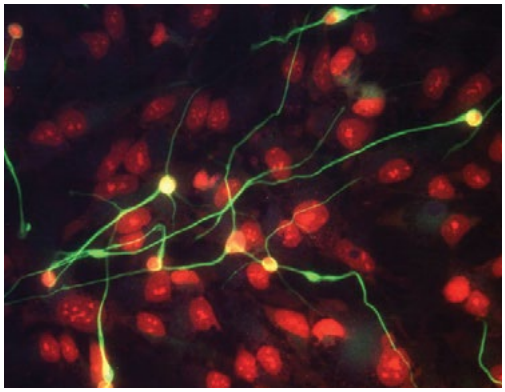
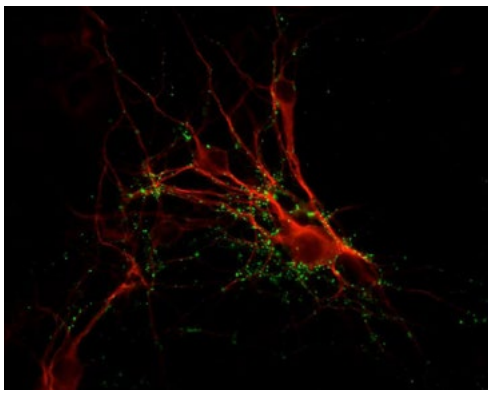


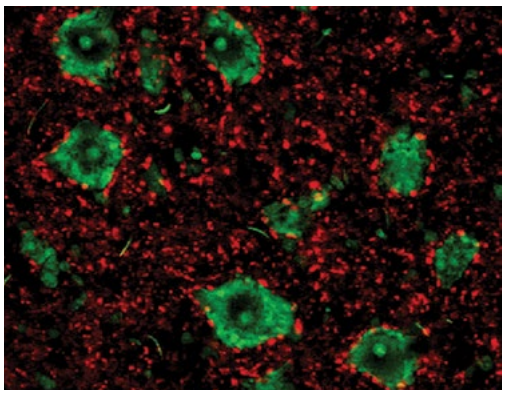
NeuN in Mouse Brain. NeuN was detected in immersion-fixed paraffin-embedded sections of mouse brain (cerebellum) using Rabbit Anti-Human/Mouse/Rat RBFOX3/NeuN Antigen Affinity-Purified Polyclonal Antibody (Novus Biologicals, Catalog # NBP1-77686). The cerebellar tissue was stained using HRP and DAB (brown), and counterstained with hematoxylin (blue).



β-III Tubulin in Differentiated Human Neural Progenitor Cells. β-III Tubulin was detected in immersion-fixed differentiated human neural progenitor cells using a Mouse Anti-Neuron-Specific β-III Tubulin (Clone TuJ-1) Monoclonal Antibody (R&D Systems, Catalog # MAB1195). The cells were stained (green) and counterstained (red).



Synapsin I in Rat Caudate Neurons. Synapsin I was detected in fixed rat caudate neurons using a Rabbit Anti-Human/Mouse/Rat Synapsin I Polyclonal Antibody (Novus Biologicals, Catalog # NB300-104). The cells were stained (green) and then counterstained for MAP proteins (red).



Synaptotagmin-1 in Rat Spinal Cord. Synaptotagmin-1 was detected in perfusion-fixed frozen sections of rat spinal cord using a Mouse Anti-Rat Synaptotagmin-1 Monoclonal Antibody (R&D Systems, Catalog # MAB43641). The tissue was stained (red) and counterstained (green).

Neuronal Markers

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Calbindin D-28K	NBP2-50048	Novus Biologicals	Human, Mouse, Rat +	4H7	WB, ICC, IHC	No
Calbindin D-28K	MAB3320	R&D Systems	Human	401025	WB, IHC, SW	No
Doublecortin (DCX)	NBP1-92684	Novus Biologicals	Human, Mouse, Rat +	3E1	WB, ICC	Yes
Enolase 2/Neuron-Specific Enolase	AF5169	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC, SW	No
MAP2	NBP1-92711	Novus Biologicals	Human, Mouse, Rat	5H11	WB, ICC, IHC	Yes
NeuroD1	AF2746	R&D Systems	Human, Mouse	Poly	WB, ICC	Yes
NF-H	NB300-135	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ELISA, IVT	No
NF-L	NB300-131	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
NF-M	NB300-133	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
RBFOX3/NeuN	NBP1-77686	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, FC	Yes
β-III Tubulin	NB100-1612	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
β-III Tubulin, Neuron-Specific	MAB1195	R&D Systems	All Species	TuJ-1	WB, ICC, IHC, SW	Yes
UCH-L1/PGP9.5	NB600-1160	Novus Biologicals	Human, Mouse, Rat +	31A3	WB, ICC, IHC, ELISA, FC, SW	Yes
UCH-L1/PGP9.5	AF6007	R&D Systems	Human, Mouse, Rat	Poly	WB, SW	No

Synaptic Markers

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Bassoon	NB120-13249	Novus Biologicals	Mouse, Rat	SAP7F407	WB, ICC, IHC, IP	No
Gephyrin/GPHN	MAB7519	R&D Systems	Human, Mouse	807423	WB, IHC	No
HOMER1	NBP1-44999	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC	No
Neuroligin 1/NLGN1	AF4340	R&D Systems	Human, Rat	Poly	WB, IHC	Yes
Piccolo	NBP1-49453	Novus Biologicals	Human, Mouse, Rat	6H9-B6	WB, ICC, ELISA	Yes
PSD-95	NB300-556	Novus Biologicals	Human, Mouse, Rat +	6G6-1C9	WB, ICC, IHC, B/N, ChIP, FC, IP	Yes
SAP102	NBP1-87691	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
Shank1	NB300-167	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Shank2	NBP2-12914	Novus Biologicals	Human, Mouse, Rat	S23b-6	WB, ICC, IHC, IP, MiAr	No
Shank2	MAB7035	R&D Systems	Human	711924	IHC	No
Shank3	NBP2-42189	Novus Biologicals	Human, Mouse, Rat	5367-51	WB, ICC, IHC	Yes
SNAP25	AF5946	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC	No
Synapsin I	NB300-104	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, IP	No
Synaptophysin	NBP2-25170	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	Yes
Synaptophysin	MAB5555	R&D Systems	Human	959904	ICC, IHC	No
Synaptotagmin 1	NBP1-91499	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
Synaptotagmin 1	MAB43641	R&D Systems	Rat	ASV30	WB, IHC, IP	No
Syntaxin 1A	AF7237	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC	No
VAMP-1	AF4828	R&D Systems	Human, Mouse	Poly	WB, IHC	No
VAMP-2	MAB5136	R&D Systems	Human, Mouse	541405	WB, IHC	No
VGLUT1/SLC17A7	NBP2-46627	Novus Biologicals	Human, Mouse, Rat	CL2754	WB, IHC	No
VIAAT/SLC32A1/VGAT	NBP2-20857	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
VIAAT/SLC32A1/VGAT	MAB6847	R&D Systems	Human	731307	ICC	No

Species Key: + Additional Species Available

Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), CyTOF (CyTOF-ready), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vivo*), IVT (*In Vitro*), MiAr (Microarray), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SCW (Single-Cell Western), SW (Simple Western™), and WB (Western blot)

Antibodies, continued

Markers for Glutamatergic Neurons

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Glutamine Synthetase	NB110-41404	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
GRIN1/NMDAR1	NB300-118	Novus Biologicals	Human, Mouse, Rat	R1JHL	WB, ICC, IHC, IP	No
GRIN2B/NMDAR2B	NB300-106	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC, IP	No
VGLUT1/SLC17A7	NBP2-46627	Novus Biologicals	Human, Mouse, Rat	CL2754	WB, IHC	No
VGLUT1/SLC17A7	MAB9054	R&D Systems	Human	732607	IHC	No
VGLUT2/SLC17A6	NBP2-46641	Novus Biologicals	Human, Mouse, Rat	CL2952	IHC	No

Markers for GABAergic Neurons

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
$GABA_B$ R1	NBP2-59336	Novus Biologicals	Human, Mouse, Rat	593A-49	WB, ICC	Yes
$GABA_B$ R1	AF7000	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC	No
$GABA_B$ R2	NBP2-59335	Novus Biologicals	Human, Mouse, Rat	S81-2	WB, ICC, IHC	Yes
$GABA_B$ R2 (N-Terminus)	AF1188	R&D Systems	Rat	Poly	WB, IHC	No
GAD1/GAD67	AF2086	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC, SW	Yes
GAD2/GAD65	NBP1-33284	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
GAD2/GAD65	AF2247	R&D Systems	Human	Poly	WB, IHC,	No
GAT-1/SLC6A1	NBP1-89802	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
VIAAT/SLC32A1/VGAT	NBP2-20857	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
VIAAT/SLC32A1/VGAT	MAB6847	R&D Systems	Human	731307	ICC	No

Markers for Dopaminergic Neurons

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Dopamine β-Hydroxylase	NBP1-31386	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
FOLR1	MAB5646	R&D Systems	Human	548908	WB, ICC, CyTOF, ELISA, FC	Yes
FOLR1	AF6936	R&D Systems	Mouse	Poly	ICC	No
HNF-3β/FoxA2	NBP2-02088	Novus Biologicals	Human, Mouse	OTI3C10	WB, ICC, IHC, FC	Yes
HNF-3β/FoxA2	AF2400	R&D Systems	Human	Poly	WB, ICC, ChIP	Yes
LMX1b	NBP2-41194	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, ELISA	No
Nurr1/NGFI-Bβ/NR4A2	NB110-40415	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC, IP	No
Nurr1/NGFI-Bβ/NR4A2	AF2156	R&D Systems	Human, Mouse	Poly	WB, IHC	No
SLC6A3/DAT1	NBP2-22164	Novus Biologicals	Mouse, Rat, Human (–ve)	mAb16	WB, ICC, IHC, ELISA, IP	Yes
Tyrosine Hydroxylase	NB300-109	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, SW	No
Tyrosine Hydroxylase	AF7566	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC, SW	No

Markers for Glycinergic Neurons

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
VIAAT/SLC32A1/VGAT	NBP2-20857	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
VIAAT/SLC32A1/VGAT	MAB6847	R&D Systems	Human	731307	ICC	No

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Species Key: + Additional Species Available

Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), CyTOF (CyTOF-ready), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vivo*), IVT (*In Vitro*), MiAr (Microarray), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SCW (Single-Cell Western), SW (Simple Western™), and WB (Western blot)

Markers for Serotonergic Neurons

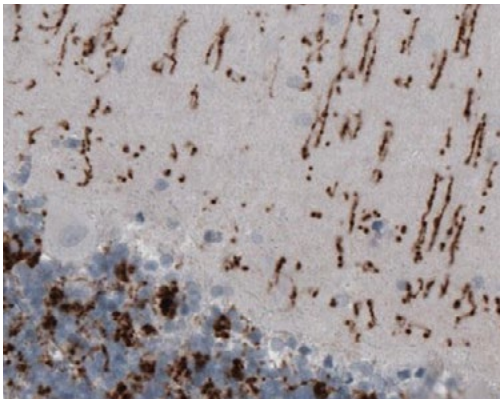
Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Dopa Decarboxylase/DCC	AF3564	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC, IHC, IP	Yes
Pet1	NBP2-55967	Novus Biologicals	Human, Mouse, Rat	Poly	ICC	No
SLACA4/5-HTTLPR/Serotonin Transporter	NBP2-57729	Novus Biologicals	Human, Mouse	Poly	ICC	No
Tryptophan Hydroxylase 1/TPH-1	NBP2-67580	Novus Biologicals	Human, Mouse, Rat	SC53-07	WB, ICC, IHC, FC, IP	No
Tryptophan Hydroxylase 1/TPH-1	AF5276	R&D Systems	Human	Poly	WB, IHC	No
VMAT2	NBP1-69750	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	Yes
VMAT2	MAB8327	R&D Systems	Human	899327	WB, IHC, CyTOF, FC	Yes

Markers for Cholinergic Neurons

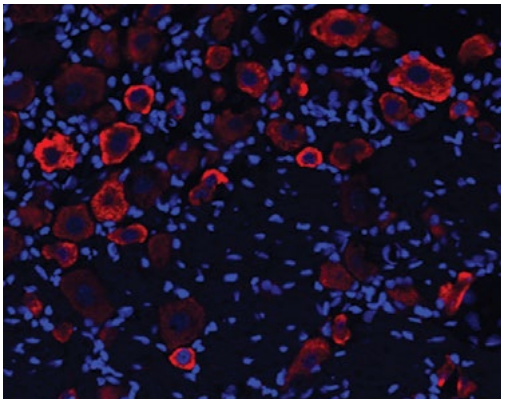
Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Acetylcholinesterase/ACHE	NB100-1519	Novus Biologicals	Human, Rat	Poly	WB, ICC, FC, PEP-ELISA	No
Choline Acetyltransferase/ChAT	NBP1-30052	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
Choline Acetyltransferase/ChAT	AF3447	R&D Systems	Human	Poly	WB, IHC	Yes
VAChT/SLC18A3	NB110-74764	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No

Markers for Motor Neurons

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
Islet-1	NBP2-33831	Novus Biologicals	Human, Mouse, Rat	Poly	ICC, IHC	No
Islet-1	AF1837	R&D Systems	Human	Poly	WB, ICC, SW	Yes
Islet-2	NBP2-57938	Novus Biologicals	Human, Mouse, Rat	Poly	ICC	No
Islet-2	AF4244	R&D Systems	Human	Poly	WB, IHC	Yes
Neurogenin-2	NBP2-41195	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, ELISA	No
Neurogenin-2	MAB3314	R&D Systems	Human, Rat	7G4	IHC	No
Olig2	NBP1-28667	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, IP	No
Olig2	AF2418	R&D Systems	Human	Poly	WB, ICC, IHC, ChIP	Yes



VGLUT2 in Human Brain. The Vesicular Glutamate Transporter 2 (VGLUT2) was detected in immersion-fixed paraffin-embedded sections of human brain (cerebellum) using a Mouse Anti-Human/Mouse/Rat VGLUT2 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-46641). The cerebellar tissue was stained using HRP and DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to glutamatergic synapses in the molecular and granular layers of the cerebellum.



$GABA_B$ R1 in Rat DRG. $GABA_B$ R1 was detected in perfusion-fixed frozen sections of rat dorsal root ganglia (DRG) using a Sheep Anti-Human/Mouse/Rat $GABA_B$ R1 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF7000). The tissue was stained using the NL557-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific staining was localized to the cell bodies of DRG neurons. All cited reagents are from R&D Systems.

Antibodies, continued

Microglia Markers - Steady State

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
AIF-1/Iba1	NB100-1028	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, PEP-ELISA	No
AIF-1/Iba1	MAB7308	R&D Systems	Human	603102	IHC	No
CD11b/Integrin αM	NB110-89474	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, FC, SCW, SW	Yes
CD11b/Integrin αM	MAB1124	R&D Systems	Mouse	M1/70	ICC, IHC, CyTOF, FC, IP	Yes
CD45 (low expression)	NB100-77417	Novus Biologicals	Human, Mouse	30-F11	WB, ICC, IHC, CyTOF, FA, FC, IP, IV	Yes
CD45 (low expression)	AF114	R&D Systems	Mouse	Poly	WB, ICC, IHC, CyTOF, FC	No
CX3CR1	NBP1-76949	Novus Biologicals	Human, Mouse, Rat	Poly	ICC, IHC, ELISA, FC	Yes
F4/80/EMR1	NB600-404	Novus Biologicals	Human, Mouse	CI-A3-1	WB, ICC, IHC, EM, FC, IP, RI	Yes
F4/80/EMR1	MAB5580	R&D Systems	Mouse	521204	ICC, IHC, CyTOF, FC	Yes
M-CSF R/CD115	NBP2-37292	Novus Biologicals	Human	6B9B9	WB, ICC, IHC, ELISA	No
M-CSF R/CD115	AF3818	R&D Systems	Mouse	Poly	ICC, WB	Yes
Mer	MAB591	R&D Systems	Mouse	108921	WB, ICC	No
TMEM119	NBP2-30551	Novus Biologicals	Human, Rat +	Poly	ICC, IHC	No

Astrocyte Markers

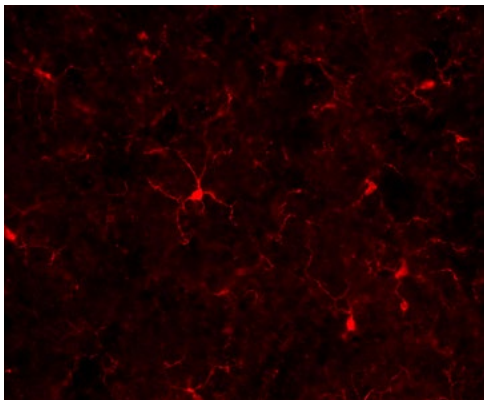
Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
A2B5	MAB1416	R&D Systems	Human, Mouse, Rat +	105	ICC, CyTOF, FC	Yes
ALDH1L1	NBP2-50045	Novus Biologicals	Human, Mouse, Rat	4A12	WB, ICC, IHC	Yes
Aldolase C	NBP2-25145	Novus Biologicals	Human, Mouse, Rat +	4A9	WB, ICC, IHC	Yes
Astrocytomas	NBP2-29820	Novus Biologicals	Human, Rat	J1–31	WB, ICC, IHC	No
Aquaporin-4	NBP1-87679	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Connexin 43/GJA1	NB100-81867	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
EAAT1/GLAST-1/SLC1A3	NB100-1869	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ELISA, FC	Yes
EAAT1/GLAST-1/SLC1A3	MAB6048	R&D Systems	Human	482420	IHC	No
EAAT2/GLT1	NBP1-20136	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC, IV	No
Galectin-3	NBP1-92690	Novus Biologicals	Human, Mouse, Rat	5C21	WB, ICC, IHC	Yes
Galectin-3	AF1197	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC, SW	Yes
GFAP	NB300-141	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, SW	No
GFAP	AF2594	R&D Systems	Human, Rat	Poly	WB, ICC, SW	Yes
Glutamine Synthetase	NB110-41404	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
HES-1	NBP1-30912	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IP	No
Notch-1	NB100-78486	Novus Biologicals	Human, Mouse, Rat (-)	mN1A	WB, ICC, IHC, CyTOF, FC, IP	Yes
Notch-1	AF1057	R&D Systems	Mouse, Rat	Poly	WB, ICC, IHC, B/N, CyTOF, FC	Yes
S100B	NBP2-45224	Novus Biologicals	Human, Mouse, Rat +	15F4N8	WB, ICC, IHC, FC	Yes
Survivin	NB500-201	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ChIP, ELISA, FC, IP, SW	Yes
Survivn	AF886	R&D Systems	Human	Poly	WB, IHC, SW	No
Thrombospondin-1	NB100-2059	Novus Biologicals	Human, Mouse, Rat +	A6.1	WB, IHC, B/N	No
Thrombospondin-1	AF3074	R&D Systems	Human	Poly	WB, IHC, SW	Yes

Species Key: + Additional Species Available

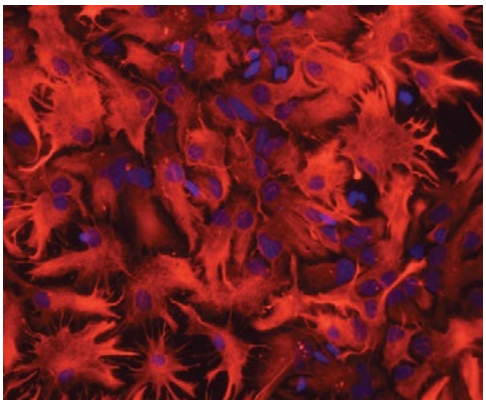
Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), CyTOF (CyTOF-ready), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vivo*), IVT (*In Vitro*), MiAr (Microarray), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SCW (Single-Cell Western), SW (Simple Western™), and WB (Western blot)

Oligodendrocyte Markers

Marker	Catalog #	Brand	Species	Clone	Applications	Conjugate
APC	NB100-91662	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC	Yes
Caspr2	AF5145	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC	Yes
CNPase	NBP2-46617	Novus Biologicals	Human, Mouse, Rat	CL2887	WB, ICC, IHC	No
MAG/Siglec-4a	NBP1-81817	Novus Biologicals	Human, Mouse, Rat	Poly	IHC	No
MAG/Siglec-4a	AF538	R&D Systems	Rat	Poly	WB, IHC	Yes
MBP	NB600-717	Novus Biologicals	Human, Mouse, Rat +	12	WB, ICC, IHC, ELISA, RI	No
MBP	MAB42282	R&D Systems	Human, Mouse, Rat	932908	WB, ICC, IHC	No
MOG	NBP2-46634	Novus Biologicals	Human, Mouse, Rat	CL2858	WB, IHC	No
MOG	AF2439	R&D Systems	Mouse	Poly	WB, IHC	Yes
Myelin PLP	NBP1-87781	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
NG2/MCSP	NBP2-66979	Novus Biologicals	Human, Mouse, Rat	JM10-13	WB, IHC, FC	No
NG2/MCSP	MAB2585	R&D Systems	Human	LHM-2	WB, IHC, CyTOF, FC	Yes
NKX2.2	MAB8167	R&D Systems	Human, Mouse, Rat	883411	WB, ICC	No
Olig1	NBP1-28666	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Olig1	MAB2417	R&D Systems	Human, Mouse	257219	WB, IHC	No
Olig2	AF2418	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC, IHC, ChIP	Yes
Oligodendrocyte Marker O1	MAB1327	R&D Systems	Human, Mouse, Rat +	O1	ICC, CyTOF, FC	Yes
Oligodendrocyte Marker O4	MAB1326	R&D Systems	Human, Mouse, Rat +	O4	ICC, CyTOF, FC	Yes
OMgp	NBP1-82483	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
PDGF Rα	NBP1-44581	Novus Biologicals	Human	16A1	ICC, CyTOF, FC, IVT	Yes
PDGF Rα	AF1062	R&D Systems	Mouse	Poly	WB, IHC, B/N	Yes
SOX10	MAB2864	R&D Systems	Human, Rat	20B7	ICC, IHC	Yes
SOX10	AF2864	R&D Systems	Human	Poly	WB, ICC	Yes



AIF-1/Iba1 in Mouse Spinal Cord. AIF-1/Iba1 was detected in perfusion-fixed sections of mouse spinal cord using a Goat Anti-Human/Mouse/Rat AIF-1/Iba1 Polyclonal Antibody (Novus Biologicals, Catalog # NB100-1028). The tissue was stained using an Alexa Fluor® 555-conjugated donkey anti-goat IgG secondary antibody (red).



GFAP in Rat Astrocytes. Glial Fibrillary Acidic Protein (GFAP) was detected in immersion-fixed rat astrocytes using a Sheep Anti-Human/Rat GFAP Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF2594). The cells were stained using the NorthernLights™ 557-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. All cited reagents are from R&D Systems.

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