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NB110-74753 Protocol

Serum protocol for Olfactory Marker Protein Antibody (NB110-74753)

Protocol specific for Olfactory Marker Protein Antibody (NB110-74753):
https://www.novusbio.com/products/olfactory-marker-protein-antibody_nb110-74753
Protocol for Paraffin Wax Embedding and IHC using Rabbit antibody to rat & mouse OMP (NB110-74753)

We used Neonatal rats just to avoid the decalcification. If you have already a protocol in place for the preparation of the Olfactory tissue, then we encourage you to try that first.
Tissue used: heads of neonatal rats of age of up to 4 days
Fixative: whole tissue fixation by immersion in 4% PFA or Zamboni's, in cold room for up to 4 days

A. Paraffin Wax Embedding
1. Tissue are fixed for up to 4 days by immersion in 4% PFA or Zamboni's, in cold room for up to 4 days
2. Place tissue sections in small plastic cages, clearly label with lead pencil.
3. Dehydration of tissue:
 a. 70% Ethanol O/N
 b. 80% Ethanol 1 Hour
 c. 90% Ethanol 1 Hour
 d. 100% Ethanol 1 Hour
 e. 100% Ethanol 1 Hour
 f. Chloroform O/N
4. Afternoon of same day, make sure there is enough wax in jug on bottom shelf of oven and in the thermal console.

5. Plastic cages with tissue inside are put into a glass jar and covered with warm wax. Leave in oven for 30 to 60 mins
6. Pour wax from step 4 into container for disposal. Cover sections again with filtered wax. Put jar into vacuum, leave for 1-1.5 hours.
7. Release vacuum pressure SLOWLY, pour wax off into container for disposal. Pour more filtered wax onto sections, place back in vacuum for another 1-1.5 hours.
8. Embedding into metal moulds:
 a. Wipe flame burner thoroughly of any leaked alcohol before lighting (top up with absolute alcohol)
 b. Turn cold plate on
 c. Wipe down hot surface with tissue or squeegy.
 d. Turn pre-heated metal moulds upside down onto the cold plate for a few seconds.
 e. Take plastic cages containing tissue sections out of jar and put in the warm wax compartment of embedding machine.
 f. Put empty jar of warm wax on top shelf of oven for reuse.
 g. Squirt a little hot wax into metal mould and place on warm surface.
 h. Drain wax off tissue section and put tissue block (flattest side down) into metal mould using metal forceps dipped in warm wax.
 i. Holding the tissue section down with forceps, place metal mould onto little cold plate for a few sections to set it level.
 j. Fill mould with warm wax from the thermal console.
 i. Label a white plastic top clearly with pencil and place on top of metal mould ensuring a good seal.
 l. Fill plastic top with warm wax until it is very full (wax will shrink when cools)
 m. Place on cold plate for approximately 30 mins
 n. Collect specimens and turn off cold plate.

B. Staining
DAY 1
Step 1: Deparaffinisation
- Incubate in Xylene (2 x 10 min), 100% ethanol (2 x 5 min), 95% , 70% ethanol, dH2O (1 x 5 min each)
Step 2: Antigen retrieval (in microwave using antigen retrieval tank & 1mM EDTA pH. 8.0)
- Pour 1mM EDTA into the tank. Boil (3 min at high power)
- Place slides in the tank (5 min at med/low power)
- Remove tank from microwave. Top up with 1mM EDTA (5 min med/low power)

- Remove tank from microwave. Allow to cool at room temperature (20 min) & transfer to 1x TBS-azide
Step 3: Elimination of endogenous peroxidase activity
- 1% H_2O_2, 50% Methanol in dH_2O (15 ml 100% Methanol + 15 ml dH_2O + 1 ml 30% H_2O_2), a few drops/slide (10 min) in the humidifying chamber.
Step 4: Flush and Wash
- Flush slides with excess 1x TBS-azide. Place slides in 1x TBS-azide (5 min)
Step 5: Blocking non-specific binding sites
- 20% NHS (without heat inactivation) in 1xTBS-azide, 250 ul/slide (1 hr) in the humidifying chamber

Step 6: Primary Antibody
- Diluted in 1% NHS - TBS-azide, 250 ul/slide (overnight) in the humidifying chamber (pour some water at the bottom of chamber to avoid drying of slides)
- Dilutions: We used a concentration of 4 ug/ml for Rabbit antibody to rat & mouse OMP (Olfactory Marker Protein): IgG (NB110-74753)
DAY 2
Step 7: Wash
- 1x TBS-azide (3 x 5 min)
Step 8: Secondary Antibody
- Biotinylated donkey anti-sheep/rabbit Antibody (Jackson Laboratories). The antibodies were dissolved in 50% glycerol in water.
- 1:500 diluted in 1% NHS - TBS-azide, 250 ul/slide (90 min) in the humidifying chamber
Step 9: Wash
- 1x TBS (3 x 5 min)
Step 10: ABC solution
- 1 drop reagent A + 1 drop reagent B in 5 ml 1x TBS (Prepare at least 30 mins before use and place on shaker), 250 ul/slide (1 hr) in the humidifying chamber
Step 11: Wash
- 1x TBS (3 x 5 min)
Step 12: DAB reaction
- 1 tablet (Sigma D-4293) dissolved in 5ml 1x TBS + 3.5 ul of 30% H_2O_2 , 250 ul/slide (until colour develops ~ 5-10 min)
- Note: Dissolve tablet in 1ml dH_2O, then top up to 5ml with 1x TBS
Step 13: Stop reaction
- Discard the DAB solution on top of the slides directly into 50% Bleach. Transfer slides into 1x TBS-azide.
Step 14: Haematoxylin Counterstaining
1. Place slides in dH_2O to rinse
2. Place in Haematoxylin for 10 sec
3. Running water for 1 min
4. Quick dip in acid alcohol for 2 sec
5. Running water for 1 min
6. Lithium carbonate for 2 min
7. Running water for 1 min
8. Absolute alcohol 1 for 10 sec
9. Absolute alcohol 2 for 10 sec
10. Absolute alcohol 3 for 10 sec
11. Xylene 1 for 2 min
12. Xylene 2 for 2 min
13. Coverslip (depex)
14. Allow to dry overnight