Product Datasheet

CD4 Antibody - BSA Free

NBP1-19371

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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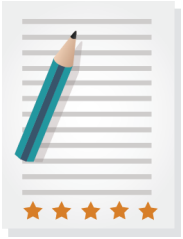
Updated 2/17/2025 v.20.1



NBP1-19371

CD4 Antibody - BSA Free

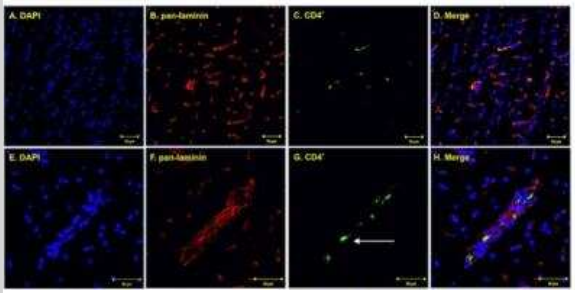
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	920
Gene Symbol	CD4
Species	Human, Mouse, Rat, Porcine, Canine, Rabbit
Reactivity Notes	Rabbit reactivity reported in the scientific literature (PMID: 22127304). Customer feedback positive on canine. Use in Porcine reported in scientific literature (PMID:33839961)
Immunogen	A synthetic peptide made to an C-terminal region of the human CD4 Antibody protein (within residues 400-458). [Swiss-Prot P01730]

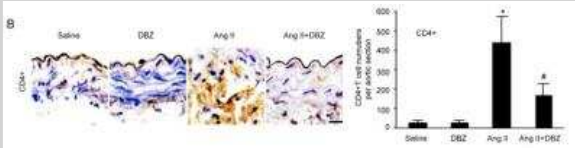
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Dual RNAscope ISH-IHC
Recommended Dilutions	Western Blot 1 ug/ml, Simple Western 1:100, Flow Cytometry 5 ug/ml, Immunohistochemistry 1:50-1:500, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:50-1:500, Immunohistochemistry-Frozen, Dual RNAscope ISH-IHC
Application Notes	This CD4 antibody is useful for ICC, Western Blot, and IHC-paraffin embedded sections. In WB, a band is seen ~45 kDa. In ICC/IF, membrane staining was observed in human brain cells. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In ICC/IF, membrane staining was observed in Jurkat cells. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

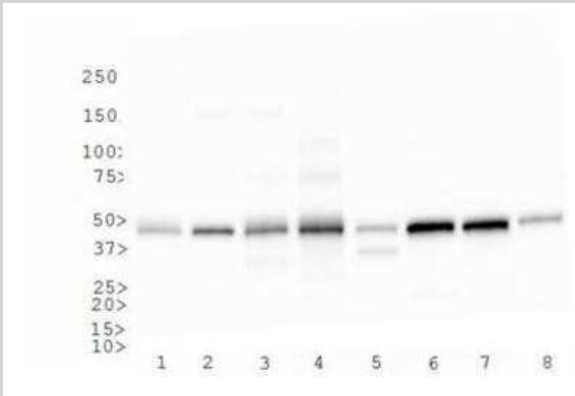
Immunohistochemistry: CD4 Antibody [NBP1-19371] - Increased CD3+ and CD4+ T-cell occurrence in the brainstem of SHR-72 transgenic rat model for tauopathies. (A-D) Immunofluorescence staining showed CD4+ T-cells in SHR-72 transgenic animals. (E- H) Immunofluorescence staining showed more perivascular than brain parenchyma infiltrating CD4+ T-cells in SHR-72 transgenic animals. PLoS One. 2019 May 23;14 (5):e0217216. doi: 10.1371/journal.pone.0217216.



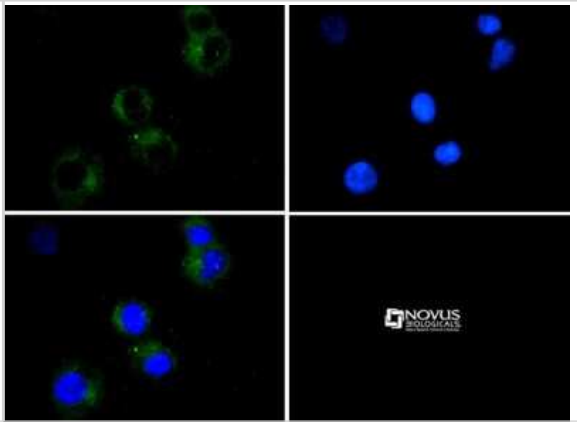
Immunohistochemistry: CD4 Antibody [NBP1-19371] - DBZ inhibits the accumulation of CD4+ T cells and Th2 differentiation in the AAAs. (B) The representation of immunohistochemical staining for CD4+ in abdominal aorta from four groups (left). Bar graphs show the percentage of CD4+ positive cell areas (right; n=3 per group). Bar: 50 um.

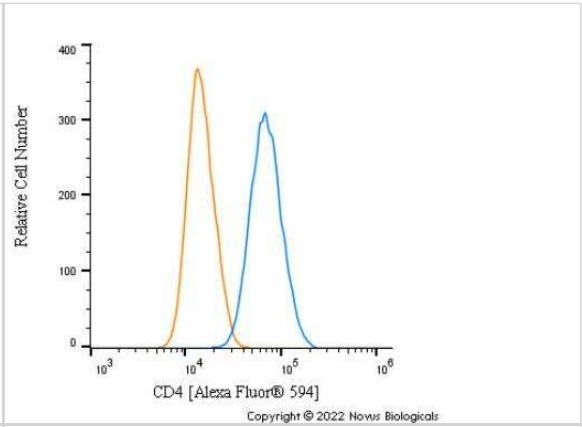
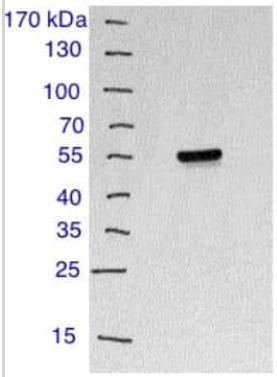
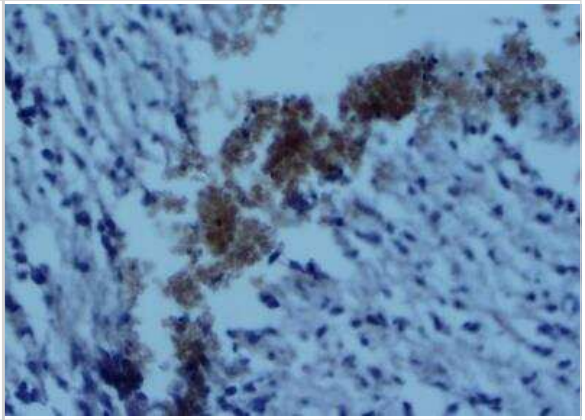
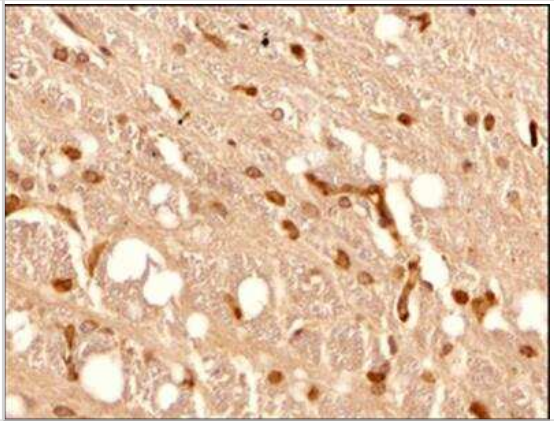


Western Blot: CD4 Antibody [NBP1-19371] - CD4 antibody was tested in the following cell lysates: 1) human spleen 2) human tonsil 3) human placenta 4) human kidney 5) human liver 6) mouse spleen 7) mouse placenta 8) rat placenta.

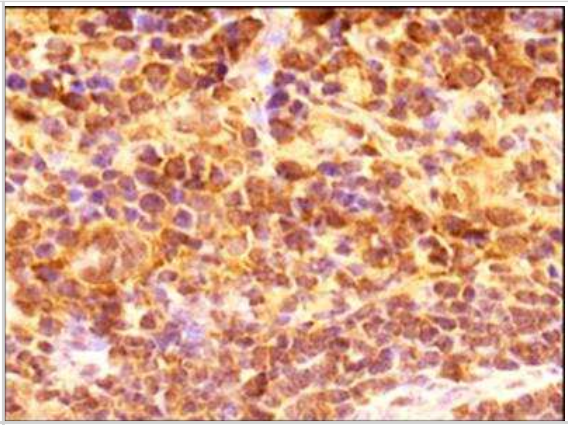


Immunocytochemistry/Immunofluorescence: CD4 Antibody [NBP1-19371] - CD4 antibody was tested in Jurkat cells with DyLight 488 (green). Nuclei were counterstained with DAPI (blue).

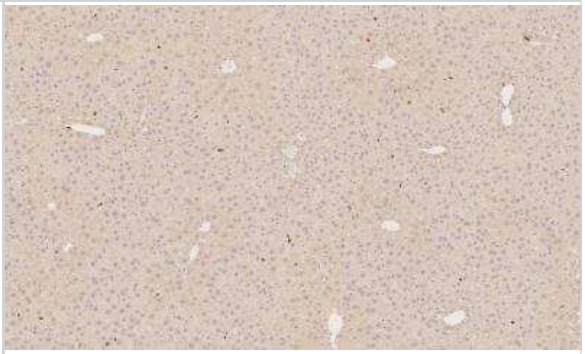


Flow Cytometry: CD4 Antibody - BSA Free [NBP1-19371] - An intracellular stain was performed on U-251 MG cells with CD4 Antibody NBP1-19371AF594 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.	
Western Blot: CD4 Antibody [NBP1-19371] - Mouse spleen, 25ug total protein. Image from verified customer review.	
Immunohistochemistry-Paraffin: CD4 Antibody [NBP1-19371] - Staining of paraffin-embedded mouse brain tissue. Photo courtesy of product review by verified customer.	
Immunohistochemistry-Paraffin: CD4 Antibody [NBP1-19371] - Analysis of a FFPE tissue section of mouse brain using CD4 antibody at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody primarily generated a strong signal in the membranes of a subset of cells in the tested section.	

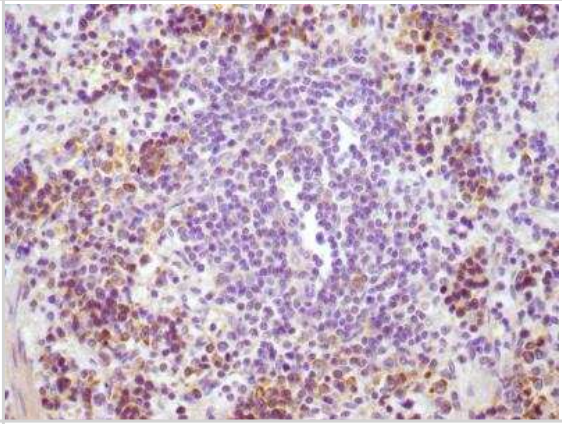
Immunohistochemistry-Paraffin: CD4 Antibody [NBP1-19371] - Analysis of a FFPE section of mouse spleen using CD4 antibody at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated a strong signal in the membranes of a subset of cells in the spleen section and the staining showed punctate appearance in some cells.



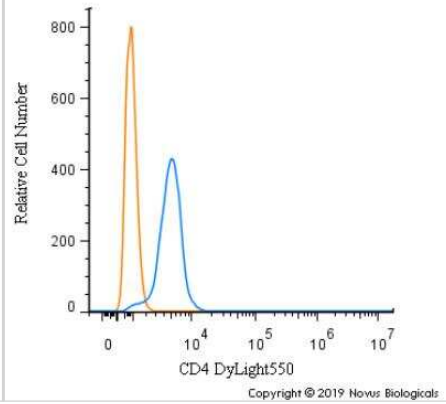
Immunohistochemistry-Frozen: CD4 Antibody [NBP1-19371] - Staining in mouse liver tissue.

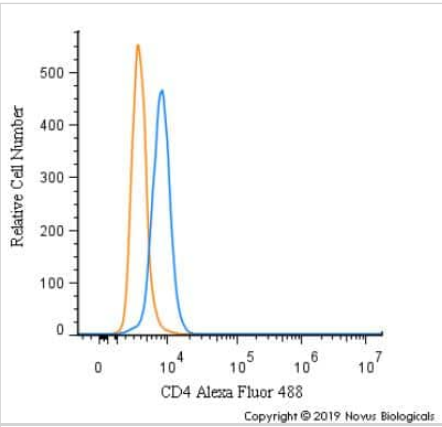
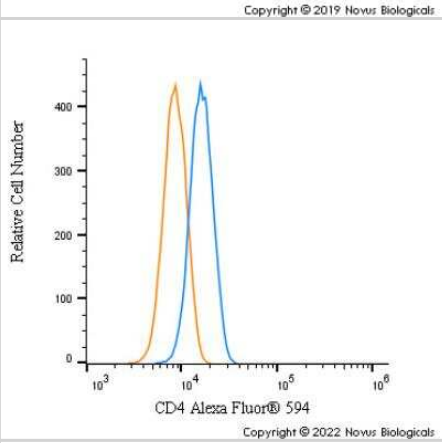
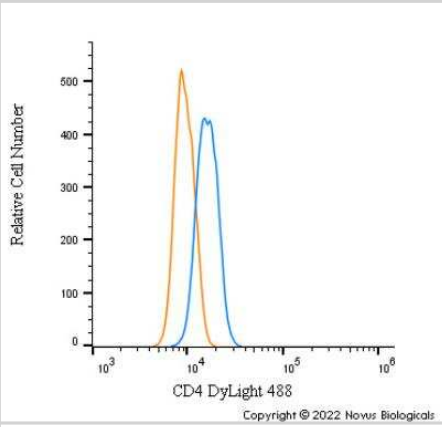
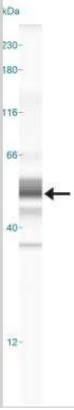


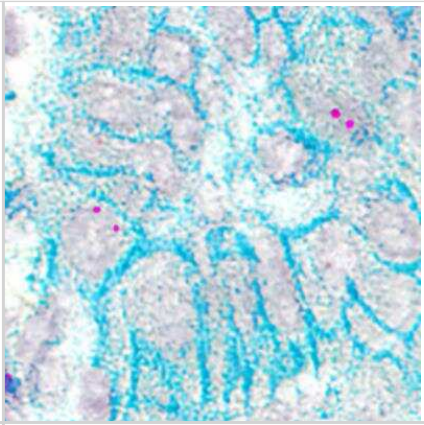
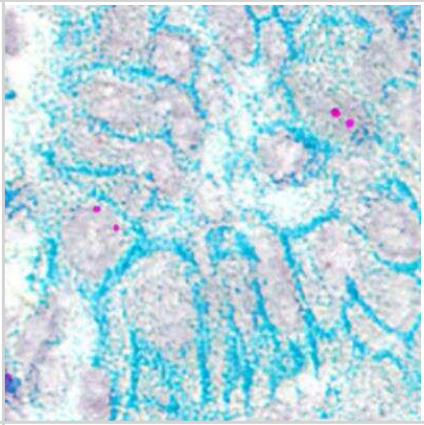
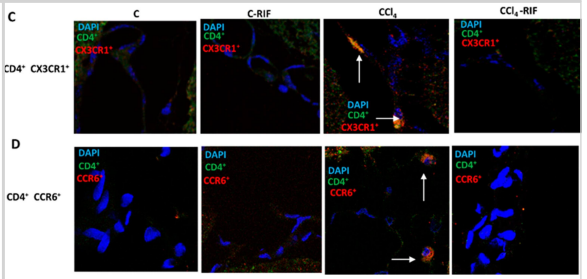
Immunohistochemistry-Paraffin: CD4 Antibody [NBP1-19371] - Alcohol-fixed paraffin-embedded rat spleen tissue. Dilution 1:150. Image from verified customer review.

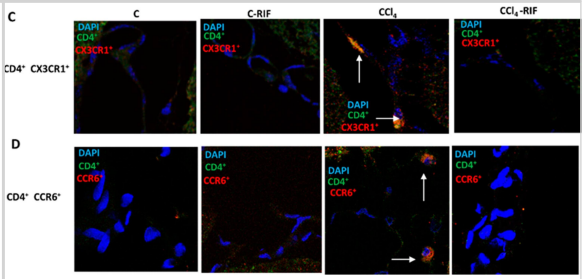


Flow Cytometry: CD4 Antibody [NBP1-19371] - An intracellular stain was performed on Jurkat cells with CD4 Antibody NBP1-19371R (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 550.



Flow Cytometry: CD4 Antibody [NBP1-19371] - An intracellular stain was performed on Jurkat cells with CD4 Antibody NBP1-19371AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	
Flow Cytometry: CD4 Antibody [NBP1-19371] - An intracellular stain was performed on U937 cells with CD4 Antibody NBP1-19371AF594 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.	
Flow Cytometry: CD4 Antibody [NBP1-19371] - An intracellular stain was performed on U937 cells with CD4 Antibody NBP1-19371G (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488.	
Simple Western: CD4 Antibody [NBP1-19371] - Lane view shows a specific band for CD4 in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Dual RNAscope ISH-IHC: CD4 Antibody [NBP1-19371] - IFNG mRNA (red) and CD4 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human lung cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAScope Probe Hs-IFNG and CD4 antibody at 1:100 dilution. Tissue was stained on Leica Bond RX using RNAscope (TM) 2.5 LS Reagent Kit-RED, BOND Polymer Refine Detection (DAB) and Hematoxylin (Cat. No. DS9800), BOND Polymer Refine Red Detection and Hematoxylin and RNAscope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).	
Dual RNAscope ISH-IHC: CD4 Antibody [NBP1-19371] - CCR5 mRNA (red) and CD4 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human lung cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAScope Probe Hs-CCR5 and CD4 antibody at 1:100 dilution. Tissue was stained on Leica Bond RX using RNAscope (TM) 2.5 LS Reagent Kit-RED, BOND Polymer Refine Detection (DAB) and Hematoxylin, BOND Polymer Refine Red Detection and Hematoxylin and RNAscope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).	
ATM activation by chloroquine alleviates senescence.(a) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in human skin fibroblasts (HSFs). A gradually increased level of p16 indicates cellular senescence, while elevated γH2AX level indicates accumulated DNA damage. (b) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in mouse embryonic fibroblasts (MEFs). (c) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in brain tissues isolated from 3-, 10-, and 18-month-old male mice. (d) SA-β-Gal staining in HSFs treated with sh-ATM or scramble shRNA. Scale bar, 100 μm. (e) Quantification of SA-β-Gal-positive staining of (d) from five views randomly captured for each group. Data represent means ± SEM. ***p<0.001. (f) Immunoblots showing increased γH2AX and unaffected LC3I/II in HSFs treated with sh-ATM or scramble shRNA. (g) Immunoblots showing protein levels of pS1981 ATM, γH2AX, and cleaved caspase-3 in HSFs treated with 10 μM of CQ for indicated time. (h) SA-β-Gal staining in HSFs expressing either scramble or ATM shRNA treated with 1 μM CQ or DMSO (12 hr). Scale bar, 100 μm. (i) Quantification of SA-β-Gal-positive staining of (h) from five views randomly captured for each group. Data represent means ± SEM. ***p<0.001; 'N.S.' indicates no significant difference. (j) HSFs at passage 20 were continuously cultured with 1 μM CQ or DMSO, and cell number was calculated at each passage. Data represent means ± SEM. ***p<0.01. (k) Immunoblots showing protein levels of γH2AX, p62, and LC3 in MEFs treated with 1 μM CQ or DMSO. Note that CQ had little effect on the expression levels of p62 and LC3. (l) MEFs at passage one were continuously cultured in 20% O2 with 1 μM CQ or DMSO, and cell number was determined at each passage. Data represent means ± SEM. ***p<0.01.10.7554/eLife.34836.006Figure 1—source data 1.Statistical analysis for SA-β-Gal positive staining.10.7554/eLife.34836.007Figure 1—source data 2.Statistical analysis for EdU positive staining.Statistical analysis for SA-β-Gal positive staining.Statistical analysis for EdU positive staining.Decline of ATM-centered DNA repair machinery during senescence.(a) Real-time PCR analysis showing progressively elevated	

mRNA level of p21 in continuously cultured human endothelial cells (HUVEC). **p<0.01. (b) SA-β-Gal staining of HUVEC cells at indicated passages. Scale bar, 100 μm. (c) HUVEC cells at P21, P18, P12, and P7 were subjected to transcriptome analysis. A minimum average rpkm value of 1.0 and maximum 10% fluctuation in young cells (P7 Vs P12) was set as the threshold. Genes were downregulated by more than 20% in pre-senescent, and senescent cells compared with young cells (P21/P18 Vs P12/P7) were selected. (d) Pathway analysis of genes identified in (c) by STRING v10. (e) Downregulation of ATM-related DNA repair genes during senescence.ATM regulates replicative senescence. (a) Representative images showing cells treated with Scramble (sh-NC) or sh-ATM. (b) Percent EdU-positive cells in sh-NC or sh-ATM treated HSFs. Views were randomly captured and at least 100 cells were included in each group. Data represent means ± SEM. ***p<0.001. (c) Immunoblots showing protein levels of pS1981 ATM and γH2AX in HSFs treated with 10 μM chloroquine (CQ) or 0.4 μM CPT (4 hr). Note that CQ activated ATM (pS1981) without increasing γH2AX, while CPT activated ATM accompanied by increased γH2AX. (d) SA-β-Gal staining in primary MEFs treated with 1 μM CQ or DMSO. Scale bar, 100 μm. (e) Quantification of SA-β-Gal-positive staining of (d) from five views randomly captured for each group. Data represent means ± SEM. ***p<0.001. (f) Percent EdU-positive cells in HSFs treated with DMSO, 1 μM or 10 μM CQ. Views were randomly captured and at least 100 cells were included in each group. Data represent means ± SEM. ***p<0.001. (g) Representative images showing proliferative HSFs treated with different doses of CQ for the indicated time points. (h) Immunoblots showing LC3B levels in HSFs treated with indicated dose of CQ for indicated period of time. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29717979), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: CD4 Antibody - BSA Free [NBP1-19371] - Rats with mild liver damage show immune cells infiltration in cerebellum at 4 weeks, which is prevented by rifaximin. Analysis of immune cells infiltration in cerebellum was performed at 4 weeks by immunohistochemistry using antibodies against (A) CD4, a marker of T lymphocytes ($F(3,8) = 4.511$, $p < 0.01$) & (B) IBA1, a marker of meningeal & perivascular macrophages ($K\text{-}W(4,10) = 27.76$, $p < 0.0001$). (C) Double immunofluorescence with anti-CD4 & anti-CX3CR1 as markers of autoreactive CD4+CD28− T lymphocytes was performed & quantified ($K\text{-}W(4,5) = 8.711$, $p < 0.05$). (D) Double immunofluorescence with anti-CD4 & anti-CCR6 as marker of Th17 lymphocytes was performed & quantified ($K\text{-}W(4,7) = 8.858$, $p < 0.05$). Number of animals in each group was added under group names. One-way ANOVA with Tukey's test (CD4+) & nonparametric Kruskal–Wallis (K-W statistic) with Dunn's test (Iba1, CD4+/CX3CR1+ & CD4+/CCR6+) was performed to compare all groups. Values significantly different from control rats are indicated by asterisks & from CCl4 rats by a. * $p < 0.05$, a $p < 0.05$. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34440206), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Zhang X, Jiang S, Zhou X et al. Human Cytomegalovirus-IE2 Affects Embryonic Liver Development and Survival in Transgenic Mouse Cellular and molecular gastroenterology and hepatology 2022-05-13 [PMID: 35569816]

Chen X, Zeng Q, Yin L et Al. Enhancing immunotherapy efficacy in colorectal cancer: targeting the FGR-AKT-SP1-DKK1 axis with DCC-2036 (Rebastinib) Cell Death Dis 2025-01-09 [PMID: 39788945]

Ko Eun Lee, Seheon Oh, Basanta Bhujel, Chang Min Kim, Hun Lee, Jin Hyoung Park, Jae Yong Kim, Chi-Feng Hung Effect of Topical Programmed Death-Ligand1 on Corneal Epithelium in Dry Eye Mouse Biomolecules 2024-01-04 [PMID: 38254668]

Thangavel H, Dhanyalayam D, Lizardo K et al. Susceptibility of Fat Tissue to SARS-CoV-2 Infection in Female hACE2 Mouse Model International Journal of Molecular Sciences 2023-01-09 [PMID: 36674830] (Western Blot, Block/Neutralize)

Thangavel H, Lizardo K, Dhanyalayam D et al. Diets Differently Regulate Tumorigenesis in Young E0771 Syngeneic Breast Cancer Mouse Model Journal of Clinical Medicine 2023-01-04 [PMID: 36675341] (Western Blot)

Ma L, Sun L, Zhao K et al. The prognostic value of TCF1+CD8+T in primary small cell carcinoma of the esophagus Cancer Science 2021-12-01 [PMID: 34657342] (Immunohistochemistry)

Jiang Y, Hong S, Zhu X et al. IL-10 partly mediates the ability of MSC-derived extracellular vesicles to attenuate myocardial damage in experimental metabolic renovascular hypertension Frontiers in Immunology 2022-09-20 [PMID: 36203611]

Nedrelow DS, Rassi A, Ajeeb B et al. Regenerative Engineering of a Biphasic Patient-Fitted Temporomandibular Joint Condylar Prosthesis Tissue engineering. Part C, Methods 2023-06-19 [PMID: 37335050]

Oswal N, Thangavel H, Lizardo K et al. Diets Differently Regulate Pulmonary Pathogenesis and Immune Signaling in Mice during Acute and Chronic Mycobacterium tuberculosis Infection Life (Basel, Switzerland) 2023-01-13 [PMID: 36676177] (IHC-P, Mouse)

Zhou X, Zhang X, Niu D et al. Gut Microbiota Induces Hepatic Steatosis by Modulating the T Cells Balance in High Fructose Diet Mice Research Square 2023-01-04 [PMID: 37095192]

Son S, Nam J, Kim AS et al. Induction of T-helper-17-cell-mediated anti-tumour immunity by pathogen-mimicking polymer nanoparticles Nature biomedical engineering 2022-12-23 [PMID: 36564626] (IHC-Fr, Mouse)

Zhang C Immune Response to Age-Related and Noise-Induced Pathogenesis in the Cochlea Int J Mol Sci 2019-06-21 [PMID: 31216722]

More publications at http://www.novusbio.com/NBP1-19371

Procedures

Western Blot protocol for CD4 Antibody (NBP1-19371)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for CD4 Antibody (NBP1-19371)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for CD4 Antibody (NBP1-19371)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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