

Product Datasheet

CD44 Antibody (8E2F3) - BSA Free NBP1-47386

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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NBP1-47386

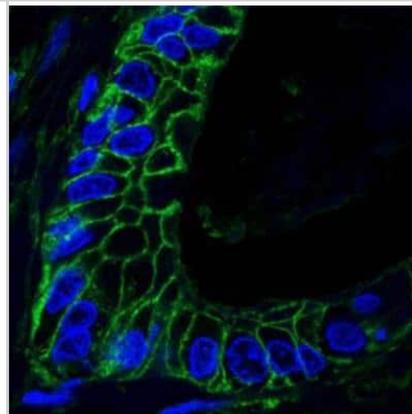
CD44 Antibody (8E2F3) - BSA Free

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	Monoclonal
Clone	8E2F3
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Ammonium sulfate precipitation
Buffer	PBS
Target Molecular Weight	82 kDa
Product Description	
Description	Novus Biologicals Knockout (KO) Validated Mouse CD44 Antibody (8E2F3) - BSA Free (NBP1-47386) is a monoclonal antibody validated for use in IHC, WB, ELISA, Flow, ICC/IF and IP. Anti-CD44 Antibody: Cited in 19 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	960
Gene Symbol	CD44
Species	Human, Mouse, Rabbit (Negative)
Reactivity Notes	Not reactive to rabbit per customer review.
Marker	Cell Membrane Marker
Immunogen	Purified recombinant fragment of human CD44 (628-699) expressed in E. coli. [Uniprot: P16070]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot, Flow Cytometry 1:200-1:400, ELISA 1:10000, Immunohistochemistry 1:200-1:1000, Immunocytochemistry/ Immunofluorescence 1:200-1:1000, Immunoprecipitation, Immunohistochemistry-Paraffin 1:200-1:1000, Flow (Intracellular), Knockout Validated Knockout validated from YCharOS Inc. (YCharOS.com)

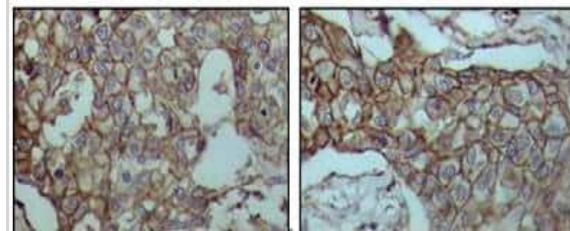


Images

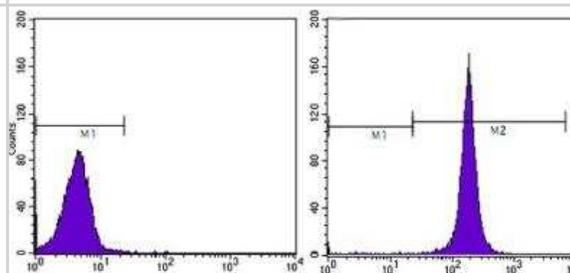
Immunocytochemistry/Immunofluorescence: CD44 Antibody (8E2F3) [NBP1-47386] - Analysis of paraffin-embedded human lung cancer tissues using anti-CD44 mAb (green), showing membrane localization. DRAQ5 fluorescent DNA dye (blue).



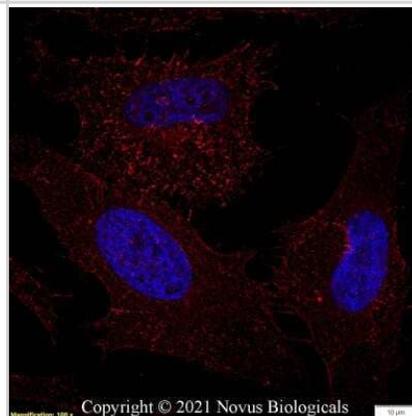
Immunohistochemistry-Paraffin: CD44 Antibody (8E2F3) [NBP1-47386] - Analysis of FFPE human breast carcinoma tissues using CD44 antibody (8E2F3). The signal was developed using DAB based detection and the sections were processed for counterstaining with hematoxylin. The antibody generated mainly a membrane staining representative of CD44 protein.



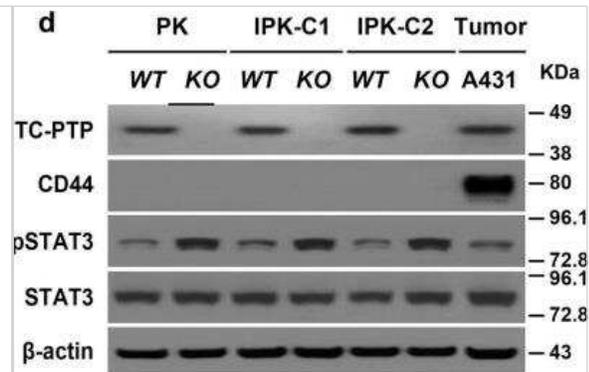
Flow Cytometry: CD44 Antibody (8E2F3) [NBP1-47386] - Analysis of HeLa cells using anti-CD44 mAb (right) and negative control (left).



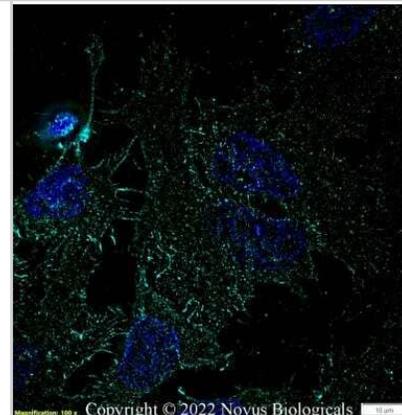
Immunocytochemistry/Immunofluorescence: CD44 Antibody (8E2F3) [NBP1-47386] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD44 Antibody [8E2F3] conjugated to DyLight 550 (NBP1-47386R) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



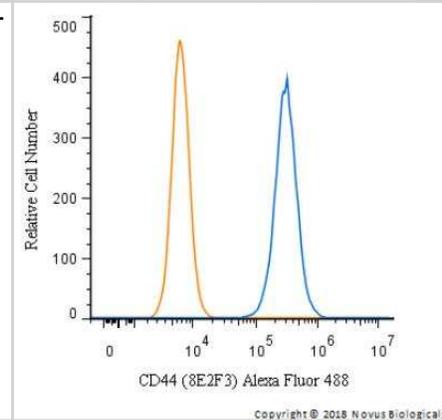
Western Blot: CD44 Antibody (8E2F3) - BSA Free [NBP1-47386] - Generation and characterization of immortalized primary keratinocytes (IPKs) from K14Cre.Ptpn2w/w and K14Cre.Ptpn2fl/fl mice. Western blot analysis of pSTAT3, STAT3, and CD44 (NBP1-47386) in primary keratinocytes and IPKs from both genotypes. The A431 cells were used as positive controls. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29955047/>) licensed under a CC-BY license.



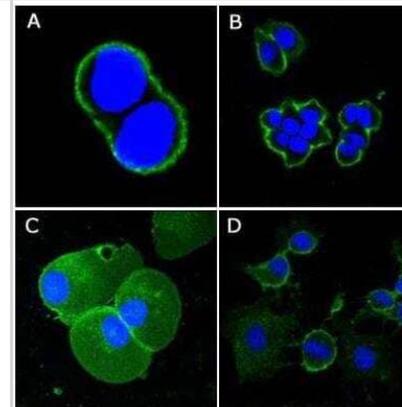
Immunocytochemistry/Immunofluorescence: CD44 Antibody (8E2F3) [NBP1-47386] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD44 Antibody [8E2F3] conjugated to Alexa Fluor 647 (NBP1-47386AF647) at 5 μ g/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



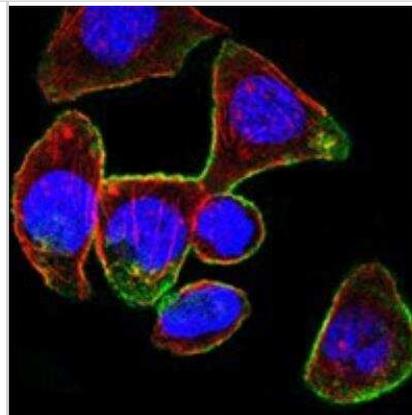
Flow Cytometry: CD44 Antibody (8E2F3) [NBP1-47386] - An intracellular stain was performed on HeLa cells with NBP1-47386AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 μ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



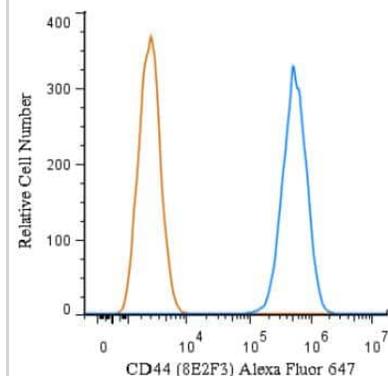
Immunocytochemistry/Immunofluorescence: CD44 Antibody (8E2F3) [NBP1-47386] - Analysis of methanol-fixed A431 (A), HeLa (B), PANC-1 (C) and EC (D) cells using anti-CD44 mAb (green), showing membrane localization. DRAQ5 fluorescent DNA dye (blue).



Immunocytochemistry/Immunofluorescence: CD44 Antibody (8E2F3) [NBP1-47386] - Analysis of PANC-1 cells using anti-CD44 mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red). DRAQ5 fluorescent DNA dye (blue).

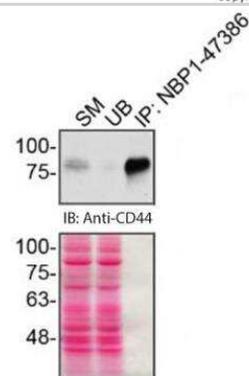


Flow (Intracellular): CD44 Antibody (8E2F3) [NBP1-47386] - An intracellular stain was performed on HeLa cells with NBP1-47386AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.

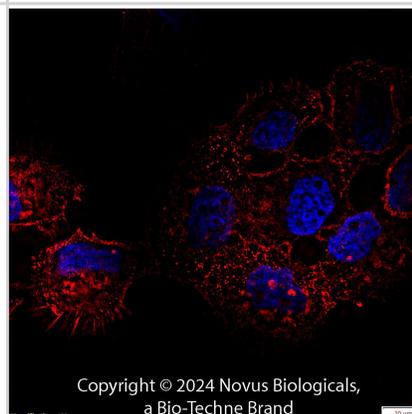


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Immunoprecipitation: CD44 Antibody (8E2F3) - BSA Free [NBP1-47386] - HAP1 lysates were prepared and immunoprecipitation was performed using 1.0 ug of the CD44 Antibody (NBP1-47386) pre-coupled to either protein G or protein A Sepharose beads. Ability of the antibodies to capture CD44 antigen was first assessed by comparing the level of CD44 antigen from the starting material (SM) to its level remaining in the unbound fractions (UB). Anti-CD44 antigen at 1/2000 was used for each immunoblot. Immunoprecipitate for CD44 Antibody (NBP1-47386) that showed depleted CD44 antigen in the UB can be seen. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).

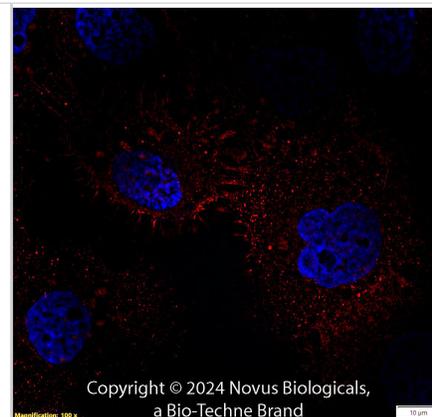


CD44 (8E2F3) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti- CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to Biotin (Catalog # NBP1-47386B) at 2 ug/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

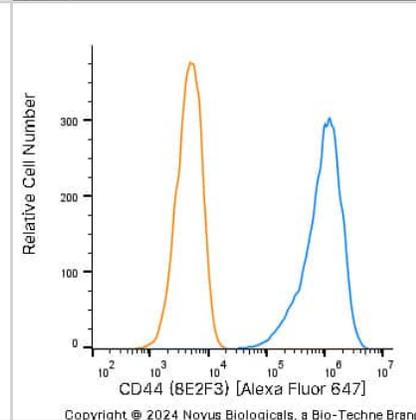


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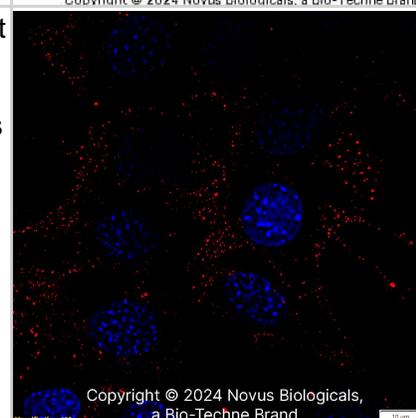
CD44 (8E2F3) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti-CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NBP1-47386R) (red) at 5 $\mu\text{g}/\text{mL}$ overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



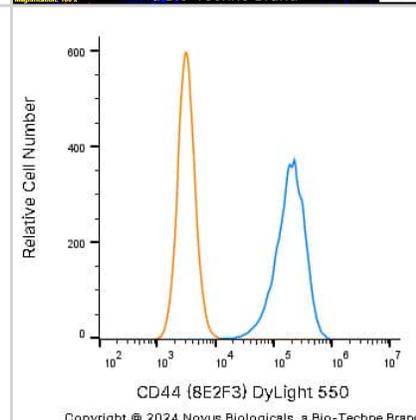
U-251 MG human glioblastoma cell line was stained with Mouse anti-CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-47386AF647, blue histogram) or matched control antibody (orange histogram).



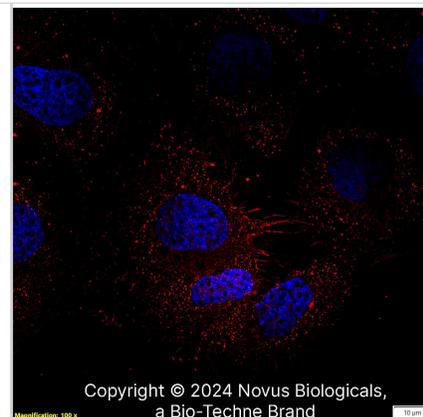
CD44 (8E2F3) was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Mouse anti- CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NBP1-47386R) (red) at 2 $\mu\text{g}/\text{mL}$ overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



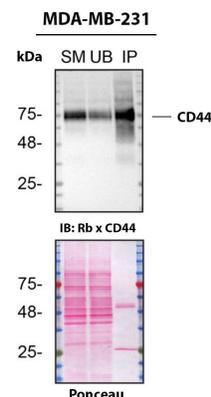
An intracellular stain was performed on A431 human skin carcinoma cell line using Mouse anti-CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NBP1-47386R, blue histogram) or matched control antibody (orange histogram) at 2.5 $\mu\text{g}/\text{mL}$ for 30 minutes at RT.



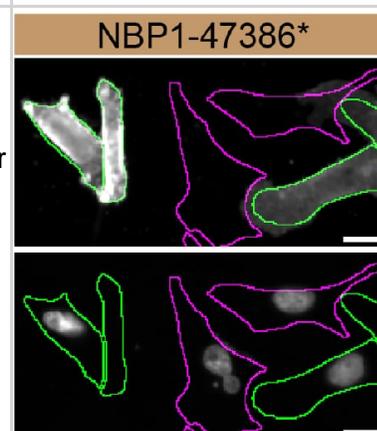
CD44 (8E2F3) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-CD44 (8E2F3) Protein-G purified Monoclonal Antibody conjugated to Biotin (Catalog # NBP1-47386B) at 5 µg/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



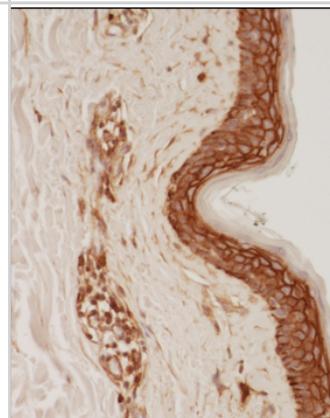
MDA-MB-231 human breast cancer cell line lysates were prepared and immunoprecipitation was performed using 2.0 µg of CD44 Antibody (8E2F3) - BSA Free (Catalog # NBP1-47386) pre-coupled to Dynabeads Protein G. Immunoprecipitated CD44 was detected in Western Blot with a rabbit CD44 antibody used at 1/3000. The Ponceau stained transfer of the blot is shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).



MDA-MB-231 human breast cancer parental cell line WT and CD44 MDA-MB-231 KO cells were labelled with a green or a far-red fluorescent dye, respectively. Cells were stained with CD44 Antibody (8E2F3) - BSA Free (Catalog # NBP1-47386) followed by incubation with an Alexa-fluor 555 conjugated secondary antibody (upper panel). DAPI-only counterstained cells shown on a lower panel. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Primary antibody dilution used: 1/1000. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).



Analysis of a FFPE tissue section of human skin using 1:200 dilution of CD44 (8E2F3) antibody. The staining was developed using HRP labeled anti-mouse secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Publications

Zuo Q, Yang Y, Lyu Y, Yang C, Chen C, Salman S, Huang T, Wicks E, Jackson W, Datan E, Qin W, Semenza G
Plexin-B3 expression stimulates MET signaling, breast cancer stem cell specification, and lung metastasis. *Cell Rep*, 2023-02-28;42(3):112164. 2023-02-28 [PMID: 36857181]

S Vondra, AL Höbler, Al Lackner, J Raffetsede, ZN Mihalic, A Vogel, L Saleh, V Kunihs, P Haslinger, M Wahrmann, H Husslein, R Oberle, J Kargl, S Haider, P Latos, G Schabbauer, M Knöfler, J Ernerudh, J Pollheimer The human placenta shapes the phenotype of decidual macrophages *Cell Reports*, 2023-01-10;42(1):111977. 2023-01-10 [PMID: 36640334]

Ning Li, Qin Zhu, Yuhua Tian, Kyung Jin Ahn, Xin Wang, Zvi Cramer, Justine Jou, Ian W. Folkert, Pengfei Yu, Stephanie Adams-Tzivelekidis, Priyanka Sehgal, Najia N. Mahmoud, Cary B. Aarons, Robert E. Roses, Andrei Thomas-Tikhonenko, Emma E. Furth, Ben Z. Stanger, Anil Rustgi, Malay Haldar, Bryson W. Katona, Kai Tan, Christopher J. Lengner Mapping and modeling human colorectal carcinoma interactions with the tumor microenvironment *Nature Communications* 2023-11-30 [PMID: 38036590]

Asare O The Role of TC-PTP-Mediated Suppression of Autophagy During Skin Carcinogenesis Thesis 2023-01-01

Salman S, Meyers DJ, Wicks EE Et al. HIF inhibitor 32-134D eradicates murine hepatocellular carcinoma in combination with anti-PD1 therapy *J Clin Invest* 2022-05-02 [PMID: 35499076] (FLOW, Mouse)

Details:

Citation using the Alexa Fluor 488 version of this antibody.

Lubanska D, Alrashed S, Mason GT et al. Impairing proliferation of glioblastoma multiforme with CD44+ selective conjugated polymer nanoparticles *Scientific reports* 2022-07-15 [PMID: 35840697] (PAGE)

Byun Js, Oh M, Lee S Et Al. The transcription factor PITX1 drives astrocyte differentiation by regulating the SOX9 gene *J. Biol. Chem.* 2020-08-05 [PMID: 32759168] (IF/IHC, Rat)

Saxena N, Bhardwaj G, Jadhav S, et al. Pre-metastatic niche drives breast cancer invasion by modulating MSC homing and CAF differentiation *bioRxiv* 2021-01-15 (ICC/IF, Human)

Li H, Chaitankar V, Zhu J et al. Olfactomedin 4 mediation of prostate stem/progenitor-like cell proliferation and differentiation via MYC *Scientific reports* 2020-12-14 [PMID: 33318499] (ICC/IF, IHC-P, Human)

Samanta D, Huang T, Shah R et al. BIRC2 Expression Impairs Anti-Cancer Immunity and Immunotherapy Efficacy *Cell Rep* [PMID: 32846130] (FLOW, Mouse)

Details:

Citation using the PE format of this antibody.

Marzban H, Sasani F Canine Mammary Gland Cancer Stem Cell and its Potential Role in Malignant Biologic Behavior *Iranian Journal of Veterinary Medicine* 2020-01-01 (WB, Canine)

Chen PY, Qin L, Li G et al. Smooth Muscle Cell Reprogramming in Aortic Aneurysms *Cell Stem Cell* 2020-04-02 [PMID: 32243809] (Mouse)

More publications at <http://www.novusbio.com/NBP1-47386>



Procedures

Western Blot Protocol for CD44 Antibody (NBP1-47386)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for CD44 Antibody (NBP1-47386)

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 (keep slides in the sodium citrate buffer at all times).

Staining:

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



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Products Related to NBP1-47386

NB800-PC1	HeLa Whole Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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