Product Datasheet

NG2/MCSP Antibody (LHM 2) - BSA Free
NB100-2688

Unit Size: 0.1 ml

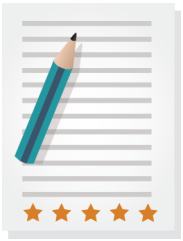
Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Publications: 13

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-2688

Updated 2/21/2025 v.20.1



NB100-2688

NG2/MCSP Antibody (LHM 2) - 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	LHM 2
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description

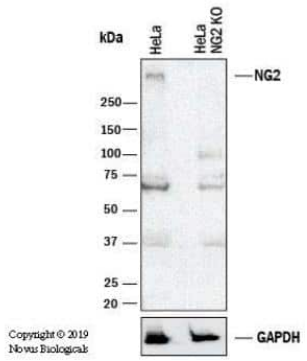
Host	Mouse
Gene ID	1464
Gene Symbol	CSPG4
Species	Human
Immunogen	A 375P cells crude extract

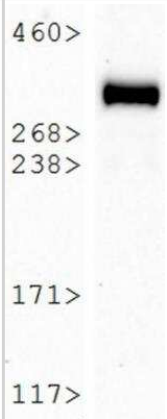
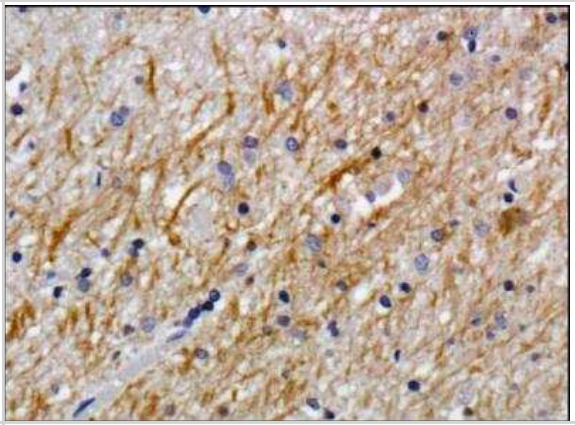
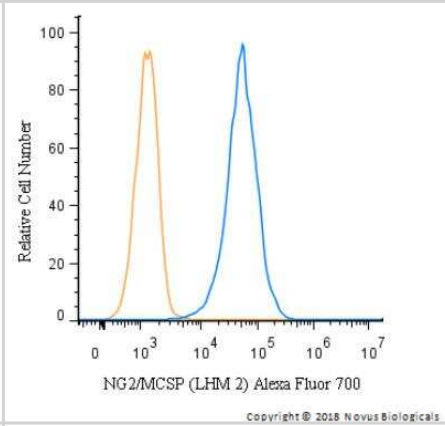
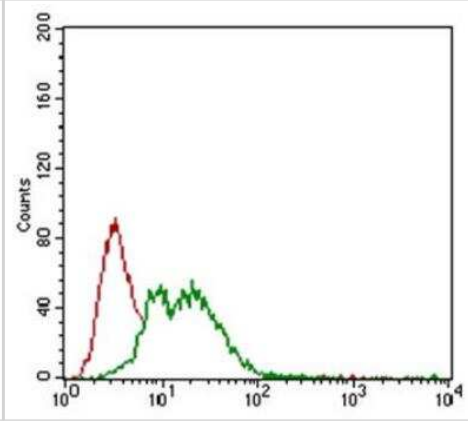
Product Application Details

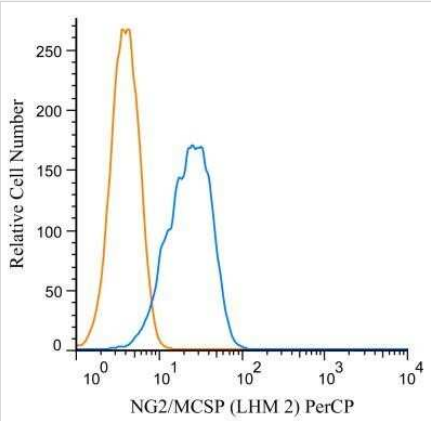
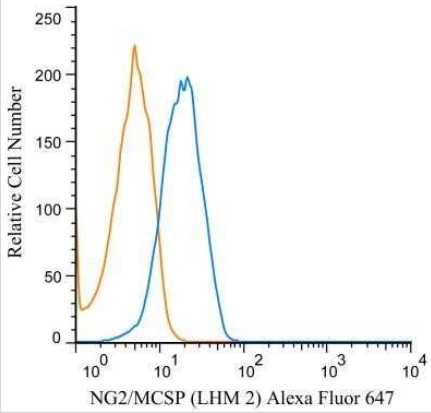
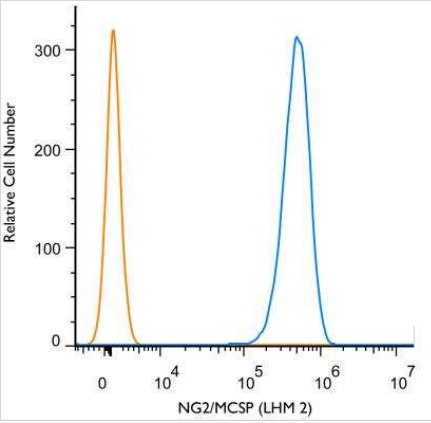
Applications	Western Blot, Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 1 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10 - 1:100, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:200, Flow (Cell Surface) 1 - 2.5 ug/mL, Knockout Validated
Application Notes	In WB a band can be seen at ~300 kDa.

Images

Western Blot: NG2/MCSP Antibody (LHM 2) [NB100-2688] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and NG2/MCSP (LHM2) knockout (KO) HeLa cell line. PVDF membrane was probed with 1.0 ug/ml of Mouse Anti-Human NG2/MCSP (LHM2) Polyclonal Antibody (Catalog # NB100-2688) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog #HAF018). Specific band was detected for NG2/MCSP (LHM2) at approximately 300 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



Western Blot: NG2/MCSP Antibody (LHM 2) [NB100-2688] - Analysis of NG2 expression in HeLa whole cell lysate.	
Immunohistochemistry-Paraffin: NG2/MCSP Antibody (LHM 2) [NB100-2688] - Staining of NG2 in human brain using DAB with hematoxylin counterstain.	
Flow Cytometry: NG2/MCSP Antibody (LHM 2) [NB100-2688] - A cell surface stain was performed on SK-MEL-28 cells with NG2/MCSP antibody (LHM 2) NB100-2688AF700 (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 5 ug/mL for 20 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 700.	
Flow Cytometry: NG2/MCSP Antibody (LHM 2) [NB100-2688] - Antibody was tested at 1:400 in MCF7 cells using an Alexa Fluor 488 secondary (shown in green) alongside unstained cells (shown in red).	

Flow (Cell Surface): NG2/MCSP Antibody (LHM 2) [NB100-2688] - A cell surface stain was performed on U87MG cells with NG2/MCSP antibody (LHM 2) NB100-2688PCP (blue) and a matched isotype control NBP2-27287PCP (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 20 minutes at room temperature. Both antibodies were conjugated to Peridinin-Chlorophyll-Protein complex.	
Flow (Cell Surface): NG2/MCSP Antibody (LHM 2) [NB100-2688] - A cell surface stain was performed on U87MG cells with NG2/MCSP antibody (LHM 2) NB100-2688AF647 (blue) and a matched isotype control NBP2-27287AF647 (orange). Cells were incubated in an antibody dilution of 2 ug/mL for 20 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Flow (Cell Surface): NG2/MCSP Antibody (LHM 2) [NB100-2688] - A surface stain was performed on A375 Cells with NG2/MCSP (LHM2) antibody NB100-2688 (blue) and a matched isotype control NBP1-43319 (orange). Cells were incubated in an antibody dilution of 1 ug/mL for 20 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).	

Publications

Monica Nanni, Dominic Rütsche, Curdin Bächler, Luca Pontiggia, Agnes S. Klar, Ueli Moehrlen, Thomas Biedermann CD146 expression profile in human skin and pre-vascularized dermo-epidermal skin substitutes in vivo Journal of Biological Engineering 2023-01-31 [PMID: 36721239]

Dey D, Tyagi S, Shrivastava V et al. Using human fetal neural stem cells to elucidate the role of the JAK-STAT cell signaling pathway in oligodendrocyte differentiation in vitro Research Square 2023-11-01 [PMID: 38227271] (ICC/IF, Human)

Stoyanov D The Role of Transcription Factor ZBTB20 in the Development of Interneurons in the Neocortex of Mice Thesis 2023-01-01

Liu HY, Koch C, Haller A Et al. Evaluation of Microfluidic Ceiling Designs for the Capture of Circulating Tumor Cells on a Microarray Platform Adv Biosyst 2020-04-16 [PMID: 32293134]

Details:
Citation using the PE version of this antibody.

Koivunotko E, Snirvi J, Merivaara A Et al. Angiogenic Potential of Human Adipose-Derived Mesenchymal Stromal Cells in Nanofibrillated Cellulose Hydrogel Biomedicines 2022-10-27 [PMID: 36289846] (WB, Human)

Details:
Citation using the Azide and BSA Free version of this antibody.

Lin Z, Zhang X, Fritch MR Et al. Engineering pre-vascularized bone-like tissue from human mesenchymal stem cells through simulating endochondral ossification Biomaterials 2022-03-08 [PMID: 35259584] (IHC-P, Human)

Details:
Citation using the Alexa Fluor 594 version of this antibody.

Li X, Guo W, Zha K et al. Enrichment of CD146+ Adipose-Derived Stem Cells in Combination with Articular Cartilage Extracellular Matrix Scaffold Promotes Cartilage Regeneration Theranostics 2019-07-09 [PMID: 31410204] (WB, Human)

Arrigoni C, Bongio M, Talo G et al. Rational Design of Prevascularized Large 3D Tissue Constructs Using Computational Simulations and Biofabrication of Geometrically Controlled Microvessels. Adv Healthc Mater. 2016-05-18 [PMID: 27191352] (ICC/IF, Human)

Kose O, Kutulola AO, Odell EW, Waseem A. Changes in the expression of stem cell markers in oral lichen planus and hyperkeratotic lesions. J Oral Sci. 2007-06-01 [PMID: 17634726] (IF/IHC, Human)

Legg J, Jensen UB, Broad S et al. Role of melanoma chondroitin sulphate proteoglycan in patterning stem cells in human interfollicular epidermis. Development. 2003-12-01 [PMID: 14573520] (ICC/IF, Human)

Kinney HC, Haynes RL, Xu G, Andiman SE, Folkerth RD, Sleeper LA, Volpe JJ. Neuron deficit in the white matter and subplate in periventricular leukomalacia. Ann Neurol;71(3):397-406. 2012-03-01 [PMID: 22451205] (IF/IHC, ICC/IF, Human)

Pierce GL, Donato AJ, LaRocca TJ, Eskurza I, Silver AE, Seals DR. Habitually exercising older men do not demonstrate age-associated vascular endothelial oxidative stress. Aging Cell;10(6):1032-7. 2011-12-01 [PMID: 21943306] (ICC/IF, Human)

More publications at http://www.novusbio.com/NB100-2688

Procedures

Western Blot Protocol for NG2/MCSP Antibody (NB100-2688)
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 1% Non-fat milk in TBST 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.

Flow (Cell Surface) Protocol for NG2/MCSP Antibody (NB100-2688)
Protocol for Flow Cytometry Cell Surface Staining
Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 15 mL conical tube and centrifuge for 4 minutes at 400 RCF.
a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.
Cell surface staining
1. Recommended: Block non-specific interactions using 0.5-1 ug of a species specific Fc-blocking reagent such as an anti-mouse CD16/CD32 antibody (NBP1-27946).
2. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined) to 100 uL of staining buffer (NBP2-26247) per sample (eg. use 1 mL of staining buffer for 10 samples).
3. Mix well and incubate at room temperature in dark for 20 minutes.
4. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
5. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
6. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
7. Incubate at room temperature in dark for 20 minutes.
8. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunocytochemistry/ Immunofluorescence Protocol for NG2/MCSP Antibody (NB100-2688)
Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NB100-2688

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NB100-2688AF647	NG2/MCSP Antibody (LHM 2) [Alexa Fluor® 647]

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

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-2688

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications