Product Datasheet

GLI-1 Antibody - BSA Free
NBP1-78259

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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NBP1-78259

GLI-1 Antibody - BSA Free

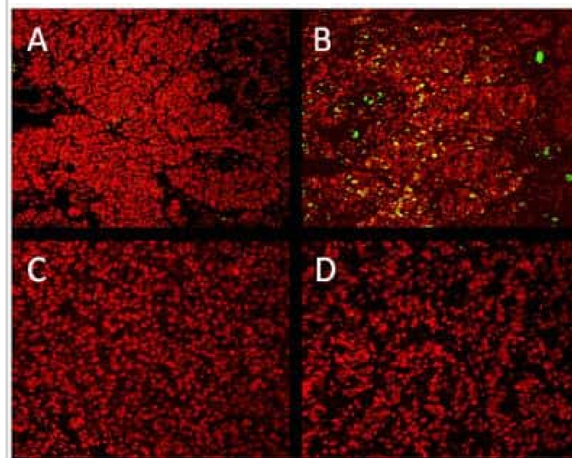
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit GLI-1 Antibody - BSA Free (NBP1-78259) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. Anti-GLI-1 Antibody: Cited in 21 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	2735
Gene Symbol	GLI1
Species	Human, Mouse
Immunogen	A synthetic peptide made to an internal portion of the human Gli1 protein (between residues 150-200) [UniProt P08151]

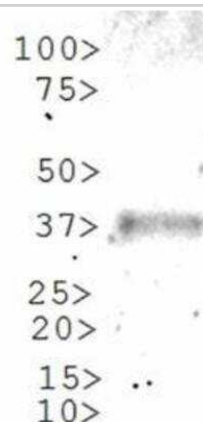
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, In-situ Hybridization
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:200, Immunohistochemistry-Paraffin 1:400, Immunohistochemistry-Frozen reported in scientific literature (PMID 25799059), In-situ Hybridization reported in scientific literature (PMID 24506883)
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

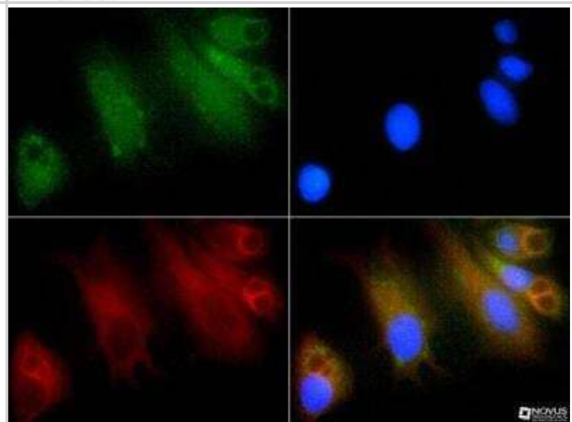
Immunohistochemistry: GLI-1 Antibody [NBP1-78259] - In vivo GLI1 expression after intratumoral administration of NVP-LDE225. LOX OMVI human melanoma cells were injected s.c into both flanks. Tumors were treated intratumorally on daily basis with vehicle (A & B) or NVP-LDE225 (C & D). Immunofluorescent microscopy of GLI1 was performed on isolated tumor tissues. GLI1 staining was performed by overnight incubation of sections at 4C with rabbit anti-GLI-1 polyclonal Ab or isotype control (A & C) followed by an 1 hr-incubation with AF488 Donkey IgG, anti-rabbit at RT (green). Counterstaining of nuclei was performed with propidium iodide (red). Pictures were taken on a confocal laser-scanning microscope system (LSM 410; Zeiss). Yellow color corresponds to double positive (anti-GLI1 and propidium iodide) nuclear staining. Image collected and cropped by CiteAb from the following publication (dx.plos.org/10.1371/journal.pone.0069064) licensed under a CC-BY license.



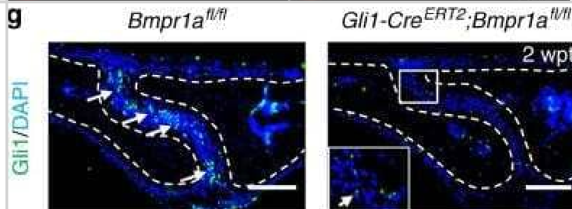
Western Blot: GLI-1 Antibody [NBP1-78259] - Analysis of GLI-1 on partial recombinant GLI-1 protein (molecular weight of partial recombinant protein is 37 kDa).

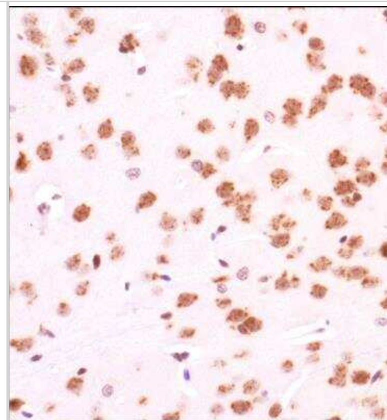
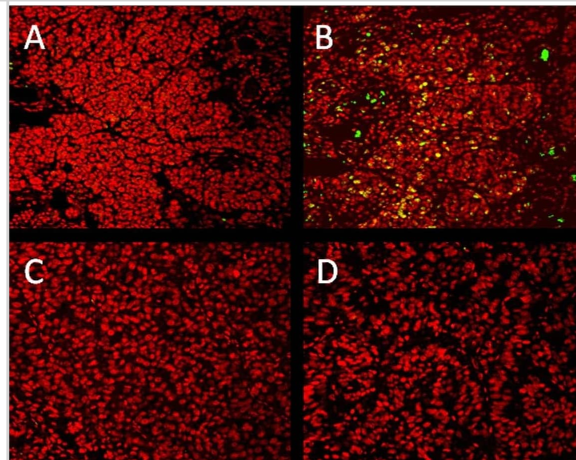


Immunocytochemistry/Immunofluorescence: GLI-1 Antibody [NBP1-78259] - GLI1 antibody was tested in HepG2 cells with FITC (green). Nuclei and actin were counterstained with DAPI (blue) and Phalloidin (red).

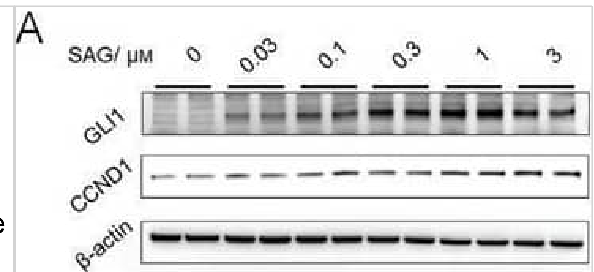


Immunohistochemistry: GLI-1 Antibody [NBP1-78259] - Immunostaining of GLI-1 (green, indicated by arrows) in the suture mesenchyme of Bmpr1-alpha-fl/fl (control) and GLI1-CreERT2; Bmpr1-alpha-fl/fl (mutant) mice 2 weeks post induction (2 wpt). The inset shows the boxed region magnified. T tests were performed. *P < 0.05; **P < 0.01. Broken lines indicate the outline of the suture. Scale bars, 100 um. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41413-018-0031-x) licensed under a CC-BY license.



Immunohistochemistry: GLI-1 Antibody [NBP1-78259] - Analysis of GLI-1 in mouse brain using DAB with hematoxylin counterstain.	
Immunocytochemistry/ Immunofluorescence: GLI-1 Antibody - BSA Free [NBP1-78259] - In vivo GLI1 expression after intratumoral administration of NVP-LDE225.LOX OMVI human melanoma cells were injected s.c into both flanks as mentioned above. Tumors were treated intratumorally on daily basis with vehicle (A & B) or NVP-LDE225 (C & D). Immunofluorescent microscopy of GLI1was performed on isolated tumor tissues. GLI1 staining was performed by overnight incubation of sections at 4°C with rabbit anti-human polyclonal Ab (B & D, NBP1-78259, Novus Biologicals, Littleton, CO) or isotype control (A & C) followed by an 1 hr-incubation with Alexa Fluor® 488 Donkey IgG, anti-rabbit (A21206, Invitrogen, Carlsbad, CA) at RT (green). Counterstaining of nuclei was performed with propidium iodide (red). Pictures were taken on a confocal laser-scanning microscope system (LSM 410; Zeiss). Yellow color corresponds to double positive (anti-GLI1 & propidium iodide) nuclear staining. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23935925), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Hedgehog signaling activation fails to promote regeneration under inflammatory conditions. A) GLI1 and CCND1 protein expression in dermal fibroblasts treated with SAG or the control vector for 2 days. B) Cell proliferation fold change in dermal fibroblasts at 24, 48, and 72 h after co□culture within RAW 264.7 cells supernatant with (sti□RAW) or without LPS stimulation and supplemented with a vector or 1 um SAG, as determined using a Cell Counting Kit□8 assay. Data presented as the mean +/- SD, n = 5 biologically independent samples. P□values were calculated using ANOVA with Tukey's multiple comparisons test. *P < 0.05. NS, not significant, P > 0.05. C) Immunofluorescence staining for Ki67 (red) and visualization of EdU (green) in dermal fibroblasts after co□culture within the supernatant of sti□RAW 264.7 cells or RAW 264.7 cells without LPS stimulation supplemented with vector or 1 um SAG for 48 h. D) Representative images and E) quantification of the results of the in vitro scratch assay of dermal fibroblasts co□cultured within the supernatant of sti□RAW 264.7 cells or RAW 264.7 cells without LPS stimulation supplemented with vector or 1 um SAG for 48 h. Dotted lines indicate the initial scratch edges. Data are presented as the mean +/- SD, n = 4. P□values were calculated using ANOVA with Tukey's multiple comparisons test. *P < 0.05. NS, not significant, P > 0.05. F) Representative images and G) quantification of tube formation in HUVECs co□cultured within the supernatant of sti□RAW 264.7 cells or RAW 264.7 cells without LPS stimulation supplemented with vector or 1 um SAG for 2 h. Data are presented as the mean +/- SD, n = 5. P□values were calculated using ANOVA with Tukey's multiple comparisons test, *P < 0.05. NS, not significant, P > 0.05. Scale bar, 200 um. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/39413023), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Mohamed FF, Ge C, Hallett SA et al. Control of craniofacial development by the collagen receptor, discoidin domain receptor 2 eLife 2023-01-19 [PMID: 36656123] (Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Human)

Shamsoon K, Hiraki D, Yoshida K et al. The Role of Hedgehog Signaling in the Melanoma Tumor Bone Microenvironment International Journal of Molecular Sciences 2023-05-16 [PMID: 37240209] (Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Human)

Juuri E, Tikka P, Domanskyi A et al. Ptch2 is a Potential Regulator of Mesenchymal Stem Cells Frontiers in Physiology 2022-04-28 [PMID: 35574464] (Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Human)

Liu Y, Zhou M, Sun J et Al. Programmed BRD9 Degradation and Hedgehog Signaling Activation via Silk-Based Core-Shell Microneedles Promote Diabetic Wound Healing Adv Sci (Weinh) 2024-10-16 [PMID: 39413023]

Donald Singer, Kristina Thamm, Heng Zhuang, Jana Karbanová, Yan Gao, Jemma Victoria Walker, Heng Jin, Xiangnan Wu, Clarissa R Coveney, Pauline Marangoni, Dongmei Lu, Portia Rebecca Clare Grayson, Tulay Gulsen, Karen J Liu, Stefano Ardu, Angus KT Wann, Shouqing Luo, Alexander C Zambon, Anton M Jetten, Christopher Tredwin, Ophir D Klein, Massimo Attanasio, Peter Carmeliet, Wieland B Huttner, Denis Corbeil, Bing Hu Prominin□1 controls stem cell activation by orchestrating ciliary dynamics The EMBO Journal 2018-12-06 [PMID: 30523147]

Jariyasakulroj S, Zhang W, Bai J et al. Ribosome biogenesis controls cranial suture MSC fate via the complement pathway in mouse and human iPSC models Stem cell reports 2023-11-06 [PMID: 37977145] (IHC-Fr, Mouse)

Details:
Dilution 1:50

Shamsoon K, Hiraki D, Yoshida K et al. The Role of Hedgehog Signaling in the Melanoma Tumor Bone Microenvironment Research Square 2023-02-17 [PMID: 33804155] (MiAr, Human)

Kuonen F, Li NY, Haensel D Et al. c-FOS drives reversible basal to squamous cell carcinoma transition Cell reports 2021-10-05 [PMID: 34610301]

Mizukoshi M, Kaku M, Thant L et al. In vivo cell proliferation analysis and cell-tracing reveal the global cellular dynamics of periodontal ligament cells under mechanical-loading Scientific reports 2021-05-07 [PMID: 33963224] (IHC-P, Mouse)

Zhang L, Zhao J, Dong J et al. GSK3 beta rephosphorylation rescues ALPL deficiency-induced impairment of odontoblastic differentiation of DPSCs Stem cell research & therapy 2021-04-06 [PMID: 33823913] (WB, Mouse)

Du J, Jing J, Yuan Y et al. Arid1a-Plagl1-Hh signaling is indispensable for differentiation-associated cell cycle arrest of tooth root progenitors Cell reports 2021-04-06 [PMID: 33826897]

Yu M, Ma L, Yuan Y, et al. Cranial Suture Regeneration Mitigates Skull and Neurocognitive Defects in Craniosynostosis Cell 2021-01-07 [PMID: 33417861] (ISH, Mouse)

More publications at http://www.novusbio.com/NBP1-78259

Procedures

Western Blot protocol specific for Gli1 antibody (NBP1-78259) WB

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 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%.

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

Immunohistochemistry-Paraffin protocol for Gli1 Antibody (NBP1-78259)

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.

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Immunocytochemistry/Immunofluorescence Protocol for Gli1 Antibody (NBP1-78259)

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.

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Products Related to NBP1-78259

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

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