

Product Datasheet

GLI-3 Antibody - BSA Free NBP2-29627

Unit Size: 0.1 mg

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

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NBP2-29627

GLI-3 Antibody - BSA Free

Product Information

Unit Size	0.1 mg
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	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	190 kDa

Product Description

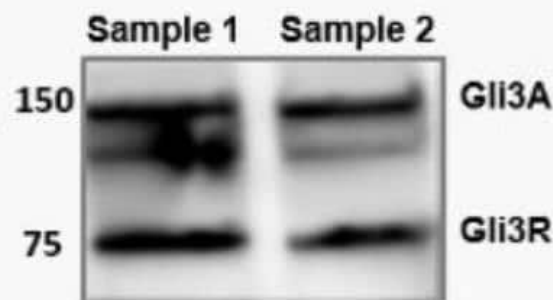
Host	Rabbit
Gene ID	2737
Gene Symbol	GLI3
Species	Human, Mouse
Reactivity Notes	Immunogen displays the following percentage of sequence identity for non-tested species: Xenopus (84%) and Chicken (85%).
Immunogen	A synthetic peptide made to an internal portion of human Gli3 protein (between residues 50-120) [UniProt P10071]

Product Application Details

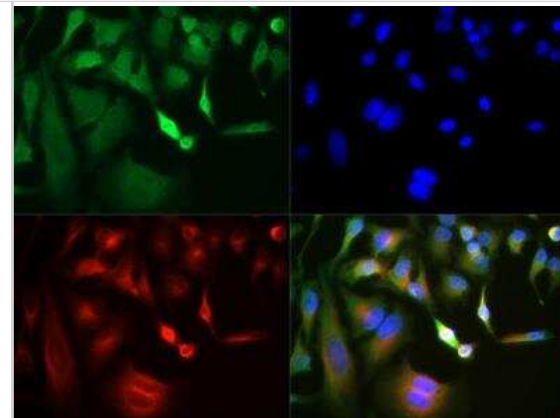
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry reported in scientific literature (PMID 26238488), Immunohistochemistry 1:100, Immunocytochemistry/Immunofluorescence 1:50, Immunohistochemistry-Paraffin 1:100, Flow (Intracellular)
Application Notes	In Western blot, bands were observed at ~75 kDa (GLI3R) in both HeLa and MEF cell lysates while an additional band was observed ~150 kDa (GLI3A) in MEF cell lysate only. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

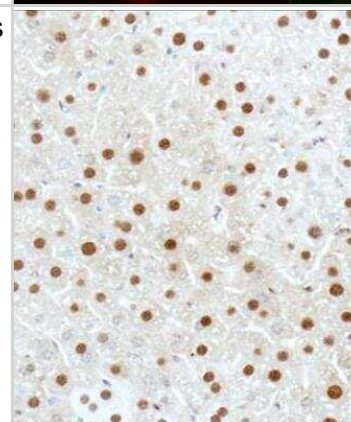
Western Blot: GLI-3 Antibody [NBP2-29627] - Adult mouse brain tissue lysates. Image from verified customer review.



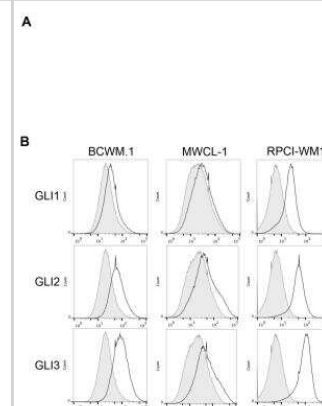
Immunocytochemistry/Immunofluorescence: GLI-3 Antibody [NBP2-29627] - HeLa cells with FITC (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



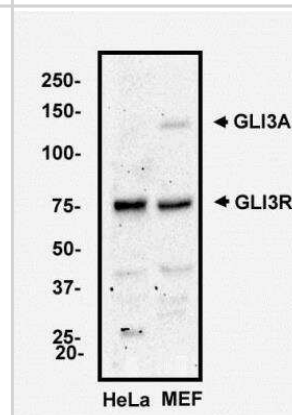
Immunohistochemistry-Paraffin: GLI-3 Antibody [NBP2-29627] - Analysis of in mouse liver.



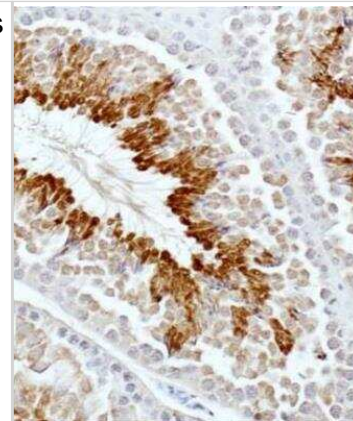
Flow (Intracellular): GLI-3 Antibody [NBP2-29627] - Intracellular staining for GLI-3 in untreated human B cell lines (BCWM.1, MWCL-1 and RPCI-WM1) using anti-GLI-3 antibody. Rabbit IgG Isotype Control (Cat# NBP2-36463) was used as a negative control. Image courtesy of Sherine Elswa, Northern Illinois University.



Western Blot: GLI-3 Antibody [NBP2-29627] - Analysis of GLI-3 in HeLa cell lysate and MEF cell lysate.



Immunohistochemistry-Paraffin: GLI-3 Antibody [NBP2-29627] - Analysis of in mouse testis.



Publications

Burleson M, Deng JJ, Qin T Et al. GLI3 Is Stabilized by SPOP Mutations and Promotes Castration Resistance via Functional Cooperation with Androgen Receptor in Prostate Cancer Molecular cancer research : MCR 2021-10-05 [PMID: 34610962] (IHC-P)

Bowers C Nutraceutical Inhibition of Hedgehog Signaling in Pancreatic Cancer Thesis 1905-07-13

Jackson DA, Smith TD, Amarsaikhan N et al. Modulation of the IL-6 Receptor alpha Underlies GLI2-Mediated Regulation of Ig Secretion in Waldenstrom Macroglobulinemia Cells. J Immunol 2015-09-15 [PMID: 26238488]



Procedures

Western blot Protocol specific for Gli3 antibody (NBP2-29627)

GLI-3 Antibody:

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 anti-Gli3 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 anti-rabbit 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 Protocol specific for Gli3 antibody (NBP2-29627)

GLI-3 Antibody:

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 4 C.
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 Gli3 Antibody (NBP2-29627)**GLI-3 Antibody:****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 1:25,000 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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Products Related to NBP2-29627

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-29627PEP	GLI-3 Antibody Blocking Peptide

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.

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