Product Datasheet

FIH-1/HIF-1AN Antibody (162c) - BSA Free
NBP1-30333

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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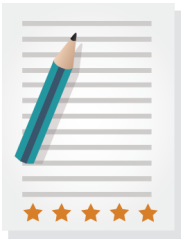
Updated 2/21/2025 v.20.1



NBP1-30333

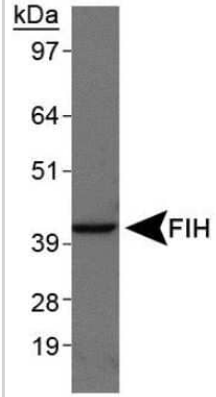
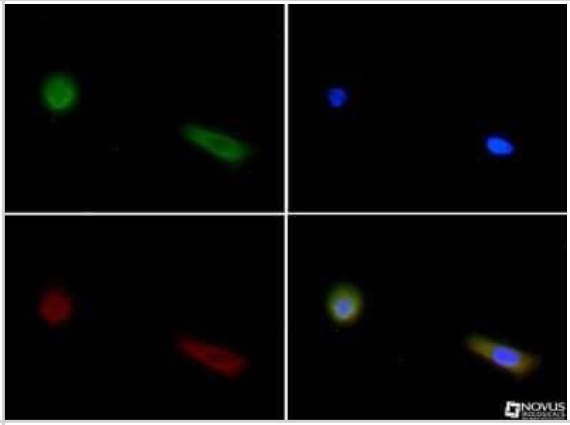
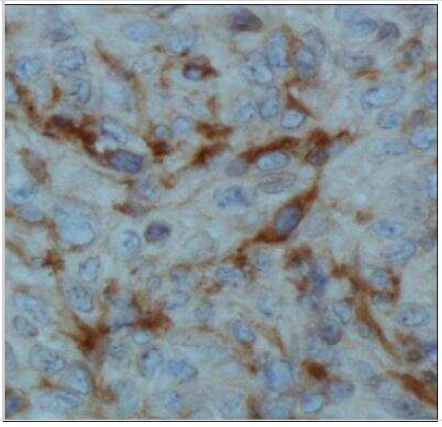
FIH-1/HIF-1AN Antibody (162c) - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	162c
Preservative	0.05% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	Tris-Glycine and 0.15M NaCl
Target Molecular Weight	40 kDa

Product Description	
Host	Mouse
Gene ID	55662
Gene Symbol	HIF1AN
Species	Human
Immunogen	Full length human Factor Inhibiting HIF-1 protein [Swiss-Prot# Q9NWT6]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 5 ug/ml, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:25, Immunohistochemistry-Paraffin 1:400
Application Notes	This Factor Inhibiting HIF-1 antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry paraffin embedded sections and Western blot analysis where a band can be seen at 40 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. 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: Factor Inhibiting HIF-1 Antibody (162c) [NBP1-30333] - Analysis in A431 cell lysates using NBP1-30333.	
Immunocytochemistry/Immunofluorescence: Factor Inhibiting HIF-1 Antibody (162c) [NBP1-30333] - FIH (162C) antibody was tested in A431 cells with FITC (green). Nuclei and alpha-tubulin were counterstained with Dapi (blue) and Dylight 550 (red).	
Immunohistochemistry: Factor Inhibiting HIF-1 Antibody (162c) [NBP1-30333] - Analysis of FIH in human renal cancer using DAB with hematoxylin counterstain.	

Publications

Ma Mingyang, Hua Shuyao, Li Gang et al. Prolyl hydroxylase domain protein 3 and asparaginyl hydroxylase factor inhibiting HIF-1 levels are predictive of tumoral behavior and prognosis in hepatocellular carcinoma. Oncotarget 2017-02-21 [PMID: 28099905] (IF/IHC, Human)

Pickel C, Gunter J, Ruiz-Serrano A et al. Oxygen-dependent bond formation with FIH regulates the activity of the client protein OTUB1 Redox Biology 2019-07-01 [PMID: 31299612] (WB, Human)

Mysore VS, Szablowski J, Dervan PB, Frost PJ. A DNA-binding Molecule Targeting the Adaptive Hypoxic Response in Multiple Myeloma Has Potent Antitumor Activity. Mol Cancer Res. [PMID: 26801054] (WB, Human)

Khan MN, Bhattacharyya T, Andrikopoulos P et al. Factor inhibiting HIF (FIH-1) promotes renal cancer cell survival by protecting cells from HIF-1alpha-mediated apoptosis Br J Cancer 2011-03-29 [PMID: 21386837] (WB, Human)

Wollenick K, Hu J, Kristiansen G et al. Synthetic transactivation screening reveals ETV4 as broad coactivator of hypoxia-inducible factor signaling. Nucleic Acids Res 2012-03-01 [PMID: 22075993] (WB, Human)

Stolze IP, Tian YM, Appelhoff RJ et al. Genetic analysis of the role of the asparaginyl hydroxylase factor inhibiting hypoxia-inducible factor (FIH) in regulating hypoxia-inducible factor (HIF) transcriptional target genes [corrected]. J Biol Chem. 2004-10-01 [PMID: 15302861] (WB, ICC/IF, Human)

Soilleux EJ, Turley H, Tian YM et al. Use of novel monoclonal antibodies to determine the expression and distribution of the hypoxia regulatory factors PHD-1, PHD-2, PHD-3 and FIH in normal and neoplastic human tissues. Histopathology. 2005-12-01 [PMID: 16324198] (IHC-P, Human)

Yan M, Rayoo M, Takano EA et al. BRCA1 tumours correlate with a HIF-1alpha phenotype and have a poor prognosis through modulation of hydroxylase enzyme profile expression. Br J Cancer;101(7):1168-74. 2009-10-06 [PMID: 19724277] (IHC-P, Human)

Cockman ME, Lancaster DE, Stolze IP, Hewitson KS, McDonough MA, Coleman ML, Coles CH, Yu X, Hay RT, Ley SC, Pugh CW, Oldham NJ, Masson N, Schofield CJ, Ratcliffe PJ. Posttranslational hydroxylation of ankyrin repeats in IkappaB proteins by the hypoxia-inducible factor (HIF) asparaginyl hydroxylase, factor inhibiting HIF (FIH). Proc Natl Acad Sci U S A;103(40):14767-72. 2006-10-03 [PMID: 17003112] (WB, Human)

Procedures

Western Blot protocol specific for FIH Antibody (NBP1-30333)
FIH-1/HIF-1AN Antibody (162c):
Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the mouse anti-FIH primary antibody (NBP1-30333) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted mouset-IgG 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 [TBS + 0.1% Tween] 3 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 (Pierce ECL).

**Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunohistochemistry-Paraffin protocol for Factor Inhibiting HIF-1 Antibody (NBP1-30333)
FIH-1/HIF-1AN Antibody (162c):
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 Factor Inhibiting HIF-1 Antibody (NBP1-30333)
FIH-1/HIF-1AN Antibody (162c):
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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NB800-PC2	Jurkat Whole Cell Lysate
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NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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