Product Datasheet

HIF-3 alpha Antibody
NB100-2529SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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Reviews: 1 Publications: 7

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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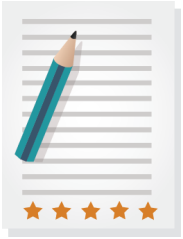
Updated 9/1/2020 v.20.1



NB100-2529SS

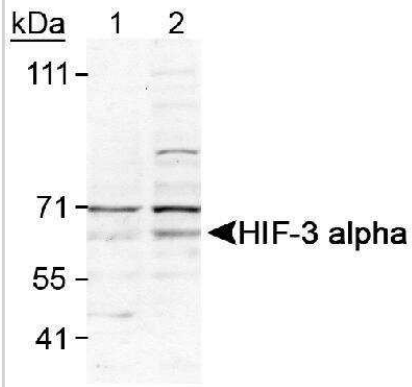
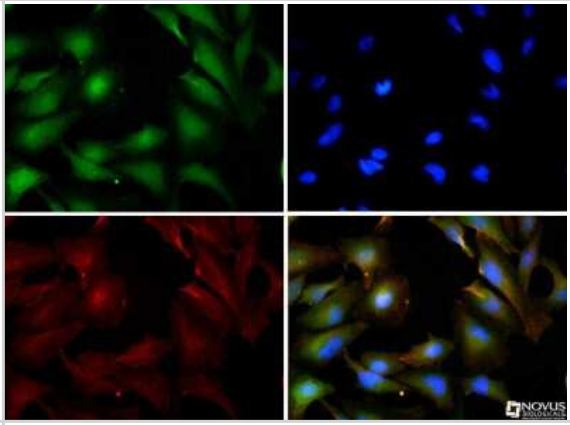
HIF-3 alpha Antibody

Product Information	
Unit Size	0.025 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Ammonium sulfate precipitation
Buffer	PBS
Target Molecular Weight	68 kDa

Product Description	
Host	Rabbit
Gene ID	64344
Gene Symbol	HIF3A
Species	Human, Mouse
Immunogen	A synthetic peptide made to an internal region of human HIF-3 alpha (within residues 250-350). [Swiss-Prot: Q66K72]

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500 - 1:1000, Flow Cytometry 1:10-1:1000, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10 - 1:500
Application Notes	In Western Blot analysis, a band is recognized at ~68 kDa representing HIF-3 alpha. Because a band at ~71 kDa is also seen in hypoxic samples, it is important to run a negative control lysate as well (see image). In ICC/IF, cytoplasmic and nuclear staining was observed in HeLa cells, which is consistent with the literature under normoxic conditions for HIF-3 alpha (PMID: 16775626).

Images

Western Blot: HIF-3 alpha Antibody [NB100-2529] - Detection of HIF-3 alpha using NB100-2529. Lane 1: Cos7 (-) control. Lane 2: Cos7 CoCl2 treated (+) control.	
Immunocytochemistry/Immunofluorescence: HIF-3 alpha Antibody [NB100-2529] - HIF3 alpha antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 549 (red).	
Western Blot: HIF-3 alpha Antibody [NB100-2529] - HIF-3a expression in papillary renal cell carcinoma samples. Image from verified customer review.	

Publications

Wang L, Cao J, Xu Q et al. 2-Dodecyl-6-Methoxycyclohexa-2,5-Diene-1,4-Dione Ameliorates Diabetic Cognitive Impairment Through Inhibiting Hif3 alpha and Apoptosis Frontiers in Pharmacology 2021-12-16 [PMID: 34975464] (WB, Mouse)

Tiwari A, Tashiro K, Dixit A Et Al. Loss of HIF1A From Pancreatic Cancer Cells Increases Expression of PPP1R1B and Degradation of p53 to Promote Invasion and Metastasis Gastroenterology 2020-08-03 [PMID: 32768595] (Mouse)

Heyboer M, Milovanova TN, Wojcik S et al. CD34+/CD45-dim stem cell mobilization by hyperbaric oxygen - Changes with oxygen dosage. Stem Cell Res 2014-03-19 [PMID: 24642336] (FLOW, Human)

Ando H, Natsume A, Iwami K et al. A hypoxia-inducible factor (HIF)-3alpha splicing variant, HIF-3alpha4 impairs angiogenesis in hypervascular malignant meningiomas with epigenetically silenced HIF-3alpha4. Biochem Biophys Res Commun 2013-02-26 [PMID: 23485455] (WB, Human)

Storti P, Bolzoni M, Donofrio G et al. Hypoxia-inducible factor (HIF)-1alpha suppression in myeloma cells blocks tumoral growth in vivo inhibiting angiogenesis and bone destruction. Leukemia 2013-01-24 [PMID: 23344526] (WB, Human)

Forooghian, F et al. Hypoxia-inducible factor expression in human RPE cells. Br J Ophthalmol;91(10):1406-10. 2007-10-01 [PMID: 17567660]

Wade, KC et al. Gene induction during differentiation of human pulmonary type II cells in vitro. Am J Respir Cell Mol Biol;34(6):727-37. 2006-06-01 [PMID: 16474099]

Procedures

Western Blot protocol for HIF-3 alpha Antibody (NB100-2529)
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.

Immunocytochemistry/Immunofluorescence Protocol for HIF-3 alpha Antibody (NB100-2529)
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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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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