

# Product Datasheet

## FIH-1/HIF-1AN Antibody - BSA Free NB100-428

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

[www.novusbio.com](http://www.novusbio.com)



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**NB100-428**

FIH-1/HIF-1AN Antibody - BSA Free

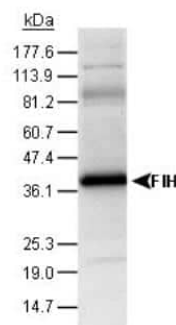
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)
Target Molecular Weight	40.3 kDa

Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rabbit FIH-1/HIF-1AN Antibody - BSA Free (NB100-428) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and IP. Anti-FIH-1/HIF-1AN Antibody: Cited in 30 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	55662
Gene Symbol	HIF1AN
Species	Human, Mouse, Rat
Reactivity Notes	Human, rat and mouse (PMID 19720742).
Immunogen	A synthetic peptide made to the C-terminal region of human Factor Inhibiting HIF-1 (between residues 300 and the C-terminus). [UniProt# Q9NWT6]

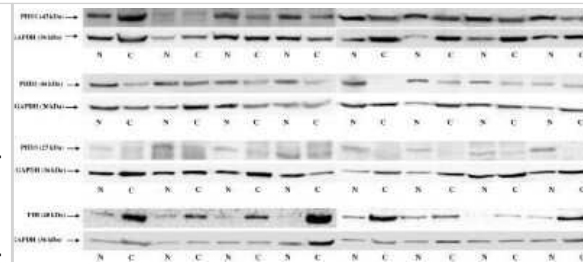
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Electron Microscopy, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000-1:5000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:50-1:200, Immunohistochemistry-Frozen 1:50-1:200, Electron Microscopy, Knockout Validated reported in scientific literature (PMID 31937588), Knockdown Validated

**Images**

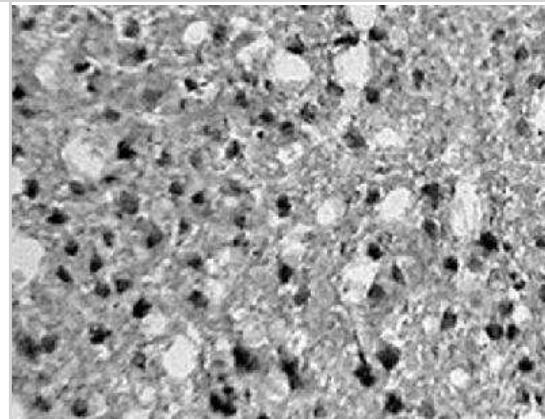
Western Blot: FIH-1/HIF-1AN Antibody [NB100-428] - Analysis of astrocytes lysate from Rat brain using FIH-1/HIF-1AN antibody at 1:500 dilution with ECL detection. The antibody detected a major band at ~40 kDa position representing FIH-1/HIF-1AN protein.



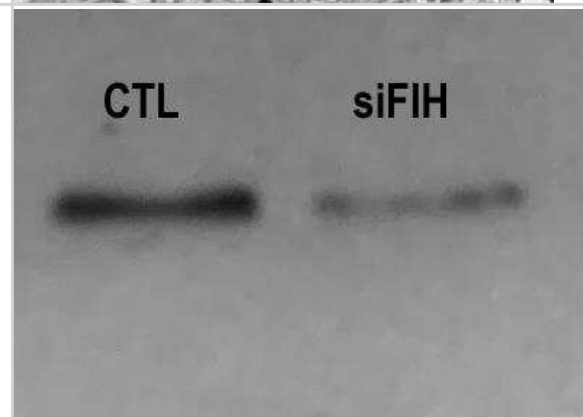
Western Blot: FIH-1/HIF-1AN Antibody [NB100-428] - Representative picture of western blot in histopathologically unchanged tissue (N) and primary cancerous tissue (C) from patients with CRC. Immunodetection of bands was performed with Rp anti- PHD1 (Catalog # NB100-310), - PHD2 (Catalog # NB100-137), - PHD3 (Catalog # NB100-139) and -FIH-1/HIF-1AN Ab (Catalog # NB100-139), followed by incubation with goat anti-rabbit HRP-conjugated Ab. The membrane was stripped and incubated with Rp anti-GAPDH Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. Image collected and cropped by CiteAb from the following publication (<https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-13-526>), licensed under a CC-BY license.



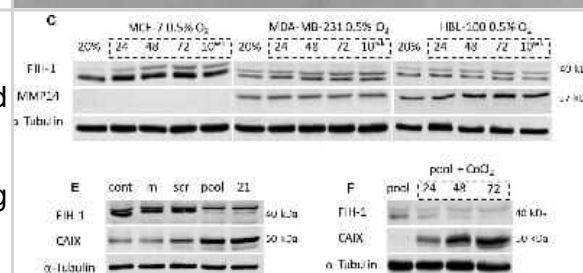
Immunohistochemistry: FIH-1/HIF-1AN Antibody [NB100-428] - Detection of FIH-1 in rat brain tissue.



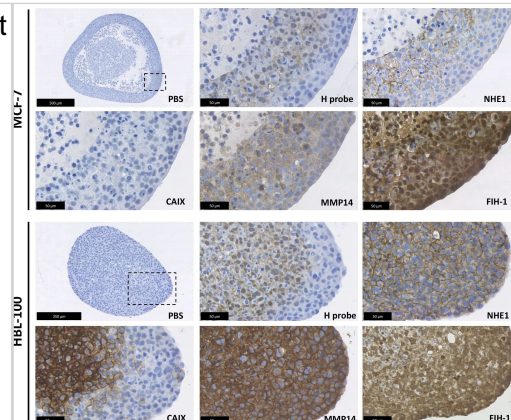
Western Blot: FIH-1/HIF-1AN Antibody [NB100-428] - Rat VSMC (vascular smooth muscle) cells treated (siFIH) or not (CTL) with a specific siRNA against FIH-1. Western blot image submitted by a verified customer review.



Western Blot: FIH-1/HIF-1AN Antibody [NB100-428] - (C) Cytoplasmic FIH-1 (lower band) and MMP14 expression levels in both aerobic and hypoxic conditions in the 3 cell lines. (E) siRNA targeting FIH-1 was used to assess whether FIH-1 had any effect on CAIX expression in MCF-7 cells cultured in 0.5% O<sub>2</sub> conditions. cont, control. m, transfection reagent alone. scr, scrambled siRNA. pool, mixture of 4 siRNAs targeting FIH-1. 21, single siRNA targeting FIH-1. (F) FIH-1 and CAIX expression levels in aerobic MCF-7 cells treated with either siRNA against FIH-1 alone, or both 400 uM CoCl<sub>2</sub> and siRNA together for 24, 48 and 72h. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28476026/>) licensed under a CC-BY license.



Immunohistochemistry: FIH-1/HIF-1AN Antibody [NB100-428] - 3D target protein expression in multicellular tumor spheroids 3D expression analysis was carried out in the MCF-7, MDA-MB-231 & HBL-100 cell lines using multicellular tumor spheroids. The spheroids were cultured in spinner flasks for 1 week before fixation. Hypoxyprobe was used to examine the formation of hypoxic areas within the spheroids. NHE1, CAIX, MMP14 & FIH-1 expression was also analyzed. Incubation for an hour with PBS instead of primary antibody acted as a control. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.17143>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Ugidos N, Mena J, Baquero S et al. Interactome of the Autoimmune Risk Protein ANKRD55 Front. Immunol. 2019-09-18 [PMID: 31620119] (Western Blot, Immunohistochemistry-Paraffin, Electron Microscopy, Immunocytochemistry/ Immunofluorescence, Rat)

Meehan J, Ward C, Turnbull A et al. Inhibition of pH regulation as a therapeutic strategy in hypoxic human breast cancer cells. Oncotarget 2017-04-17 [PMID: 28476026] (Western Blot, Immunohistochemistry-Paraffin, Electron Microscopy, Immunocytochemistry/ Immunofluorescence, Rat)

Williams AL, Khadka VS, Anagaran MCT, Lee K et Al. miR-125 family regulates XIRP1 and FIH in response to myocardial infarction Physiol Genomics 2020-07-28 [PMID: 32716698]

Vera Schützhold, Jan Gravemeyer, Anne Bicker, Thomas Hager, Claudia Padberg, Jana Schäfer, Anna Wrobeln, Melanie Steinbrink, Seher Zeynel, Thomas Hankeln, Jürgen Christian Becker, Joachim Fandrey, Sandra Winning Knockout of Factor-Inhibiting HIF ( Hif1an ) in Colon Epithelium Attenuates Chronic Colitis but Does Not Reduce Colorectal Cancer in Mice. Journal of immunology (Baltimore, Md. : 1950) 2022-03-07 [PMID: 35121641]

E G, Sun B, Liu B et al. Enhanced BPGM/2,3-DPG pathway activity suppresses glycolysis in hypoxic astrocytes via FIH-1 and TET2 Brain research bulletin 2022-11-08 [PMID: 36334804] (WB, Human)

### Details:

Dilution used in WB 1:1000

Li ZL, Wang B, Lv LL et al. FIH-1-modulated HIF-1 alpha C-TAD promotes acute kidney injury to chronic kidney disease progression via regulating KLF5 signaling Acta pharmacologica Sinica 2021-03-03 [PMID: 33658705]

Chung Y, Saitoh Y, Hayashi T et al. Mint3 is dispensable for pancreatic and kidney functions in mice Biochemistry and biophysics reports 2020-12-01 [PMID: 33319072] (WB)

Docherty C K, Nilsen M et al. Influence of 2-Methoxyestradiol and Sex on Hypoxia-Induced Pulmonary Hypertension and Hypoxia-Inducible Factor-1-alpha. J Am Heart Assoc 2019-05-03 [PMID: 30819028] (WB, Human)

Mader J, Huber J, Bonn F et al. Oxygen-dependent asparagine hydroxylation of the ubiquitin-associated (UBA) domain in Cezanne regulates ubiquitin binding J. Biol. Chem. 2020-01-14 [PMID: 31937588] (WB, KO, Human)

Pillai MR, Mihi B, Ishiwata K et al. Myc-induced nuclear antigen constrains a latent intestinal epithelial cell-intrinsic anthelmintic pathway PLoS ONE 2019-02-26 [PMID: 30807587] (WB, Mouse)

Bullen JW, Tchernyshyov I, Holewinski RJ et al. Protein kinase A-dependent phosphorylation stimulates the transcriptional activity of hypoxia-inducible factor 1 Sci Signal 2016-05-31 [PMID: 27245613] (WB, Human)

Nakaoka HJ, Hara T, Yoshino S et al. NECAB3 Promotes Activation of Hypoxia-inducible factor-1 during Normoxia and Enhances Tumourigenicity of Cancer Cells Sci Rep 2016-03-07 [PMID: 26948053] (IP, Human)

More publications at <http://www.novusbio.com/NB100-428>

## Procedures

### Western Blot protocol specific for Factor Inhibiting HIF-1 Antibody (NB100-428)

1. (30-40ug) of protein were separated via gel electrophoresis (10-20%) under denaturing conditions and transferred to nitrocellulose membrane.
2. Membrane was blocked overnight (4C) in TBS/Tween-20 (TBST) + 5% milk (blocking buffer).
3. Membrane was incubated with primary anti-FIH [cat# NB 100-428] (1:500) in blocking buffer for 2 hours at room temp.
4. Membrane was washed 3 times with TBST. 10 minutes each wash.
5. Membrane was incubated with HRP conjugated Donkey anti-rabbit secondary in blocking buffer for 2 hours at room temp.
6. Membrane was washed 3 times with TBST. 10 minutes each wash.
7. Membranes were developed for visualization using Super Signal(R) West Pico Chemiluminescent Substrate and captured on Hyperfilm ECL (Amersham Biosciences).

### Immunohistochemistry protocol for FIH-1/HIF-1AN Antibody (NB100-428)

1. Prepared 18 micron frozen rat brain tissue sections (post fixed with 10% formalin for 30 minutes).
2. Dilute the primary antibody 1:100 and incubated with diluted FIH antibody (cat# NB 100-428) at 4C, overnight.
3. Diluted the secondary antibody, biotinylated anti-rabbit IgG, according to the manufacturer's guidelines and incubated overnight at 4C.
4. Diluted the tertiary antibody, HRP-conjugated streptavidin, according to the manufacturer's guidelines and incubated overnight at 4C.
5. Visualized with DAB oxidation by HRP.





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### **Products Related to NB100-428**

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NBP3-11855	NIH 3T3 CoCl <sub>2</sub> treated/Untreated Cell Lysate
NB100-428PEP	FIH-1/HIF-1AN Antibody Blocking Peptide
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

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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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