Product Datasheet

HIF-1 alpha Antibody - BSA Free
NB100-654

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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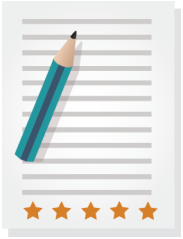
Updated 2/17/2025 v.20.1



NB100-654

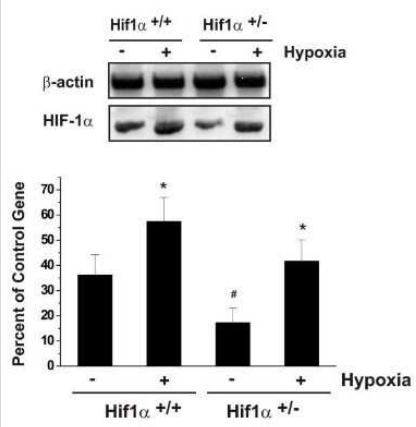
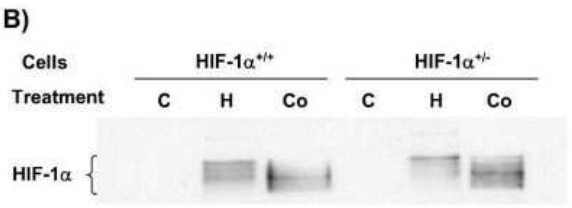
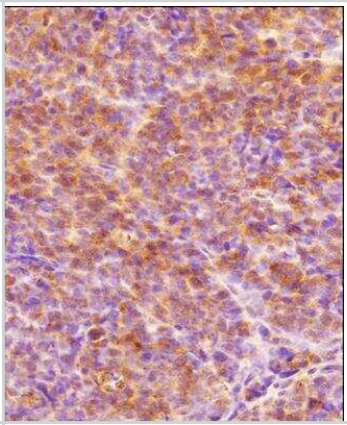
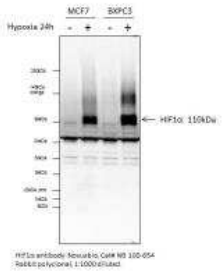
HIF-1 alpha Antibody - BSA Free

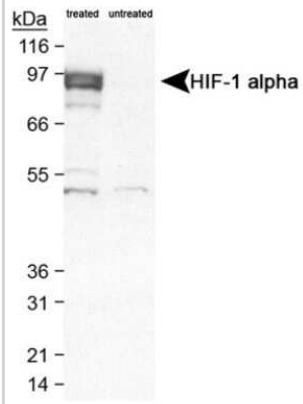
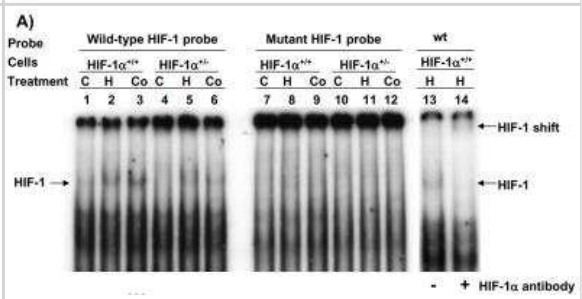
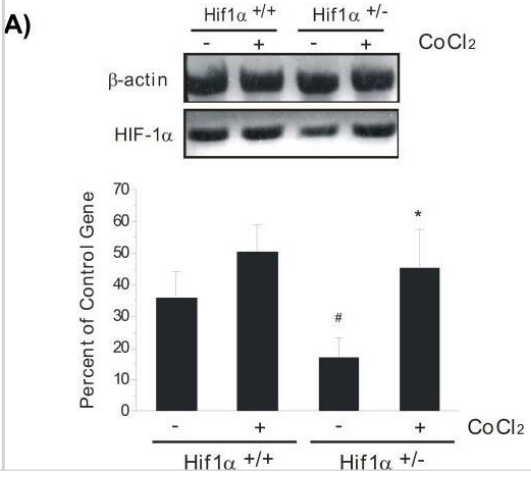
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	93 kDa

Product Description	
Host	Rabbit
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Porcine, Bovine
Reactivity Notes	Mouse reactivity was reported in scientific literature (PMID: 23959856). Rat reactivity was reported in scientific literature (PMID: 24122166). Use in Bovine reported in scientific literature (PMID:32054096).
Immunogen	This HIF-1 alpha Antibody was developed against a fusion protein containing amino acids 432 - 528 of human HIF-1 alpha [Uniprot# Q16665].

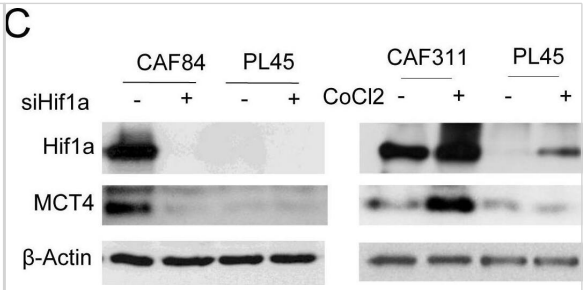
Product Application Details	
Applications	Western Blot, Simple Western, Gel Super Shift Assays, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:100, Immunohistochemistry 1:100 - 1:300, Immunocytochemistry/ Immunofluorescence 1:10 - 1:500. Use reported in scientific literature, Immunohistochemistry-Paraffin 1:100 - 1:300. Use reported in scientific literature, Immunohistochemistry-Frozen 1:100 - 1:300. Use reported in scientific literature, Gel Super Shift Assays, Knockdown Validated reported in scientific literature (PMID 32054096)
Application Notes	Nuclear extracts are recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Hypoxic HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

Western Blot: HIF-1 alpha Antibody [NB100-654] - Effects of in vitro hypoxia on the expression of mouse HIF-1 alpha in HIF-1 alpha+/- and HIF-1 alpha+/+ astrocytes. Confluent astrocyte monolayers of both cell types were exposed to a 6 h in vitro hypoxia. HIF-1 alpha mRNA expression was determined by RT-PCR as described in Materials and Methods. Each bar represents the mean +/- SD of relative density/volumes of the bands on film negatives from at least three experiments. Asterisks and number sign indicate significant difference ($p < 0.01$; one-way ANOVA, followed by multiple comparisons among means). Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-4-12), licensed under a CC-BY license.	
Western Blot: HIF-1 alpha Antibody [NB100-654] - Effects of CoCl2 treatment on HIF-1 alpha expression in mouse astrocytes. Western blots using nuclear proteins show that both hypoxia (H) and CoCl2 (Co) up-regulated HIF-1 alpha protein in HIF-1 alpha+/+ and HIF-1 alpha+/- cells. There was no HIF-1 alpha protein detected in control cells. Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-4-12), licensed under a CC-BY license.	
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody [NB100-654] - IHC analysis of a formalin-fixed paraffin-embedded tissue section of human endometrium carcinoma AN3CA cell line based xenograft using rabbit polyclonal HIF-1 alpha antibody NB100-654 at 1:300 dilution. The signal was developed using HRP-labelled secondary antibody and DAB reagent, and the section was further counterstained using hematoxylin. The tested section depicted mainly a diffused cytoplasmic staining but there were some cells which showed nuclear signal also (representing hypoxic cells).	
Western Blot: HIF-1 alpha Antibody [NB100-654] - HIF1-alpha analysis using NB100-654. Image submitted from verified customer review.	

Western Blot: HIF-1 alpha Antibody [NB100-654] - Detection of HIF-1 alpha in cobalt chloride treated/untreated COS-7 nuclear extracts using NB100-654.	
Simple Western: HIF-1 alpha Antibody [NB100-654] - Simple Western lane view shows a specific band for HIF-1 alpha in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Gel Super Shift Assays: HIF-1 alpha Antibody [NB100-654] - Mouse HIF-1 alpha+/+ and HIF-1 alpha+/- cells were exposed to hypoxia or 125 uM CoCl2 for 6 h, nuclear extracts were then prepared and EMSA carried out. HIF-1 isolated from hypoxia or CoCl2-treated cells bound to the wild-type probe (lanes #1-6) but not to the mutant probe (lanes #7-12). HIF-1/DNA complex was detected in hypoxia (H)-or CoCl2 (Co)-treated cells (lanes #2, 3, 5, 6) but not in control (C) cells (lanes #1, 4). More complex (darker band) was seen in hypoxia-or CoCl2-treated HIF-1 alpha+/+ cells (lanes #2, 3) than that in hypoxia-or CoCl2-treated HIF-1 alpha+/- cells (lanes #5, 6). Supershift assay showed that the HIF-1/DNA complex was shifted up in the presence of wild-type (wt) oligo probe and 4 ug HIF-1 alpha antiboby (lanes #13 & 14). Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-4-12), licensed under a CC-BY license.	
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-654] - Effects of CoCl2 treatment on HIF-1α expression in mouse astrocytes. (A) The cells were incubated in the presence or absence of 125 µM CoCl2 for 6 hr. HIF-1α mRNA expression was determined by RT-PCR. Each bar represents the mean ± SD of relative density/volumes of the bands on film negatives from at least three experiments. Asterisk & number sign indicate significant difference compared to relevant controls ($p < 0.01$; one-way ANOVA, followed by multiple comparisons among means). (B) Western blots using nuclear proteins show that both hypoxia (H) & CoCl2 (Co) up-regulated HIF-1α protein in HIF-1α+/+ & HIF-1α+/- cells. There was no HIF-1α protein detected in control cells (C). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17474992), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-654] - Aberrant HIF1α expression in PDAC cancer associated fibroblastsA. The level of MCT4 was determined in the indicated established pancreatic cancer cell lines & CAF cultures by immunoblotting. B. The level of MCT1 was determined in the indicated established pancreatic cancer cell lines & CAF cultures by immunoblotting. C. HIFα & MCT4 levels were determined by immunoblotting following the knockdown of HIF1α with RNAi or the induction of hypoxia through the use of CoCl2. D. The stability of HIF1α was evaluated in CAFs following treatment with cycloheximide. E. HIF1α was detected by immunoblotting in the presence of cycloheximide in the absence or presence of the proteasome inhibitor MG132. F. HIF1α protein levels were determined in the absence & presence of DMOG. G. RNA levels of the indicated genes as determined by microarray analyses (**p < 0.01 for tumor cell line vs. CAF). H. MG132 was used to interrogate the synthetic rate of HIF1α protein in the indicated cell lines. I. The expression of HIF1α target genes was evaluated in the indicated cell lines by microarray analysis (**p < 0.01 for tumor cell vs. CAF). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27623078), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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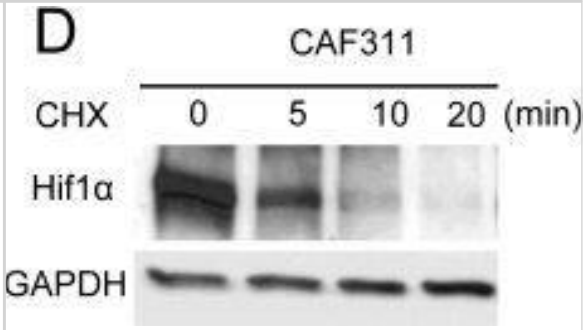

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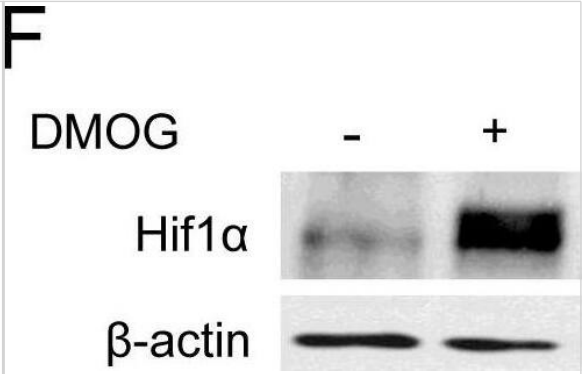

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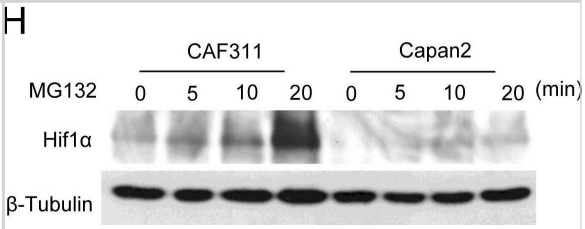

Publications

Fang T, Cao X, Wang L et Al. Bioresponsive and immunotherapeutic nanomaterials to remodel tumor microenvironment for enhanced immune checkpoint blockade Bioact Mater 2023-11-03 [PMID: 38026439]

Schilcher I, Stadler JT, Lechleitner M, et al. Endothelial Lipase Modulates Paraoxonase 1 Content and Arylesterase Activity of HDL International journal of molecular sciences 2021-01-13 [PMID: 33450841]

Michael Holzer, Sabine Kern, Ruth Birner-Grünberger, Sanja Curcic, Akos Heinemann, Gunther Marsche Refined purification strategy for reliable proteomic profiling of HDL 2/3 : Impact on proteomic complexity Scientific Reports 2016-12-05 [PMID: 27917957]

Schilcher I, Ledinski G, Radulovic S et al. Endothelial lipase increases antioxidative capacity of high-density lipoprotein Biochim Biophys Acta Mol Cell Biol Lipids 2019-06-17 [PMID: 31220617]

Strassheim D, Karoor V, Nijmeh H et al. c-Jun, Foxo3a, and c-Myc Transcription Factors are Key Regulators of ATP-Mediated Angiogenic Responses in Pulmonary Artery Vasa Vasorum Endothelial Cells Cells 2020-02-11 [PMID: 32054096] (KD, WB, Bovine)

Gericke A, Mann C, Zadeh JK et al. Elevated Intraocular Pressure Causes Abnormal Reactivity of Mouse Retinal Arterioles Oxid Med Cell Longev 2019-12-29 [PMID: 31976030] (IF/IHC, Mouse)

Zadeh, JK;Ruemmler, R;Hartmann, EK;Ziebart, A;Ludwig, M;Patzak, A;Xia, N;Li, H;Pfeiffer, N;Gericke, A; Responses of retinal arterioles and ciliary arteries in pigs with acute respiratory distress syndrome (ARDS) Exp. Eye Res. 2019-04-22 [PMID: 31022399] (IHC-Fr, Porcine)

Serganova I, Cohen IJ, Vemuri k et al. LDH-A regulates the tumor microenvironment via HIF-signaling and modulates the immune response. PLoS ONE. 2018-09-24 [PMID: 30248111] (IHC-P, Mouse)

Bouchard G, Therriault H, Geha S et al. Radiation-induced lung metastasis development is MT1-MMP-dependent in a triple-negative breast cancer mouse model. Br J Cancer 2017-02-14 [PMID: 28103615] (Mouse)

Knudsen ES, Balaji U, Freinkman E et al. Unique metabolic features of pancreatic cancer stroma: relevance to the tumor compartment, prognosis, and invasive potential. Oncotarget. 2016-11-29 [PMID: 27623078] (IF/IHC, Human)

De Bruycker S, Vangestel C, Van den Wyngaert T et al. Baseline [(18)F]FMISO microPET as a Predictive Biomarker for Response to HIF-1alpha Inhibition Combined with 5-FU Chemotherapy in a Human Colorectal Cancer Xenograft Model. Mol Imaging Biol. 2016-01-04 [PMID: 26728163] (IHC-P, Human)

Mojsilovic-Petrovic J, Callaghan D, Cui H et al. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes. J Neuroinflammation 2007-01-01 [PMID: 17474992]

More publications at http://www.novusbio.com/NB100-654

Procedures

Western Blot protocol for HIF-1 alpha Antibody (NB100-654)

Western Blot Protocol

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. 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% non-fat dry milk in TBS for 1 hour.

6. Dilute the rabbit anti-HIF-1 alpha primary antibody (NB 100-654) in blocking buffer and incubate 2 hours at room temperature.

7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.

9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).

10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

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

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Products Related to NB100-654

NBP2-36452	HeLa Hypoxic / Normoxic Cell Lysate
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

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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