Product Datasheet

HIF-1 alpha Antibody
NB100-123SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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Updated 6/15/2014 v.20.1

NB100-123SS

HIF-1 alpha Antibody (H1alpha67)

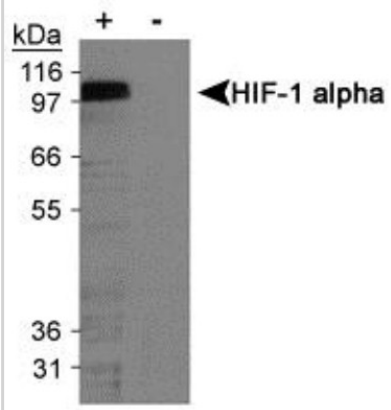
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Monoclonal
Clone	H1alpha67
Preservative	0.02% Sodium Azide
Isotype	IgG2b
Purity	Protein A purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Avian, Bovine, Canine, Ferret, Primate, Porcine, Sheep
Species Reactivity	Recognizes human, mouse, rat, bovine, monkey, porcine, sheep, canine, bird and ferret HIF-1 alpha.
Immunogen	Fusion protein containing amino acids 432-528 of human HIF-1 alpha. [UniProt# Q16665]

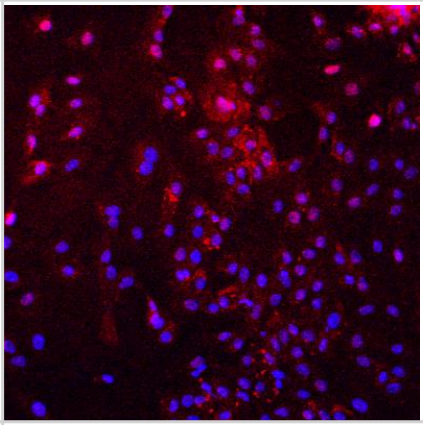
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence 1:100-1:500, Immunohistochemistry 1:100-1:300, Immunohistochemistry-Paraffin 1:100-1:300, Immunoprecipitation 1:10, Western Blot 1:500-1:1000
Application Notes	This HIF-1 alpha (H1alpha67) antibody is useful for Western blot, Immunohistochemistry on paraffin-embedded sections, Immunocytochemistry/Immunofluorescence and Immunoprecipitation. Chromatin Immunoprecipitation, Gel Super Shift Assays, and ELISA were reported in scientific literature. By Western blot, this antibody recognizes bands at 120kDa representing HIF-1 alpha in induced tissues and cells. Multiple bands may be seen at 120kDa representing post-translational modifications. Nuclear extracts are recommended for Western blot.

Images

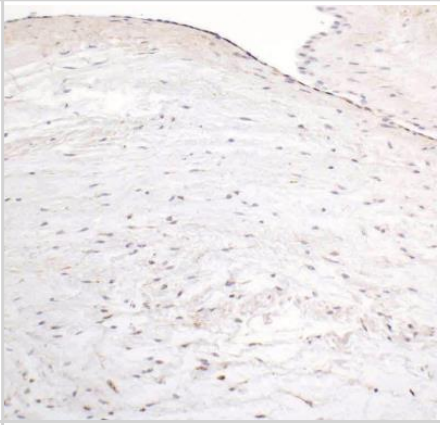
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Detection of HIF-1 alpha in cobalt chloride treated and untreated COS-7 nuclear extracts.



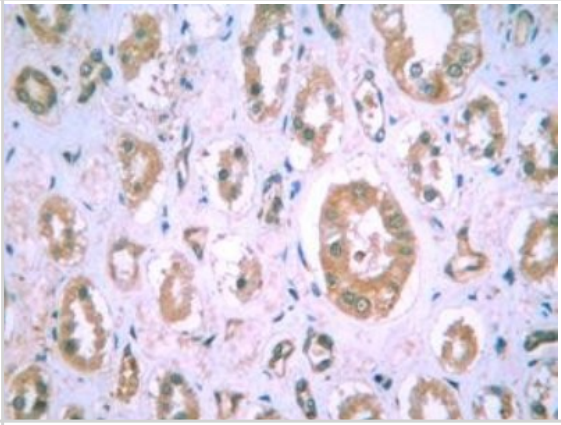
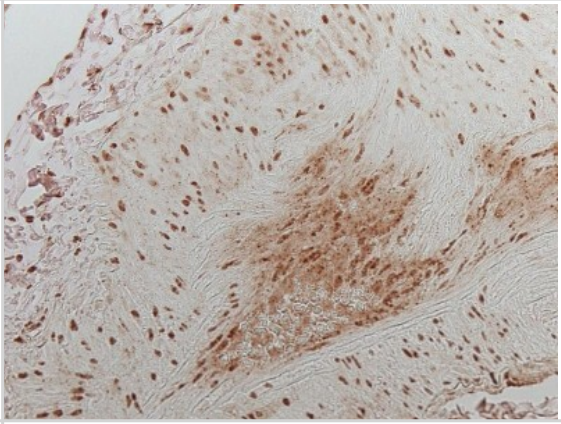
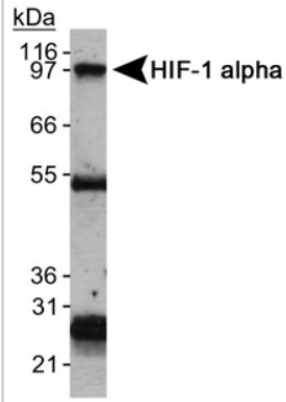
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HIF staining in pig endothelial cells under hipoxia condition. Image from verified customer review.



Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HIF-1 staining on pig tissue (brown). Image from verified customer review.



Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - On day 1, MEF cells (+/+,-/-), were grown on 15 cm dish (2x10 to the 6th cells). On day 2, cells were exposed to hypoxia for 4hrs. Cells were washed with ice cold PBS twice and whole cell protein was extracted with RIPA buffer fortified with protease. Upon quantification, 100ug of protein was fractionated on 7% polyacralymide gel. Gel was transferred overnight onto nitrocellulose membrane. The membrane was probed with HIF-1 alpha monoclonal antibody at a 1:500 dilution (NB100-123). The secondary antibody was conjugated with HRP and was used at a 1:2500 dilution. Photo courtesy of Dr. Gregg Semenza, Johns Hopkins University.

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining of HIF-1 alpha in human kidney.	
Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis of HIF-1a in human lung tissue. Image courtesy of product review by Aneta Gandjeva.	
Immunoprecipitation: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Heavy and light chains are also detected.	

Publications

Bruns Dr, Brown Rd, Stenmark Kr et al. Mitochondrial Integrity in a neonatal Bovine Model of Right Ventricular Dysfunction. Am. J. Physiol. Lung Cell Mol. Physiol. 2014 Nov 21 [PMID: 25416385]

Su Ej, Xin H, Yin P et al. Impaired fetoplacental angiogenesis in growth-restricted fetuses with abnormal umbilical artery Doppler velocimetry is mediated by aryl hydrocarbon receptor nuclear translocator (ARnT). J. Clin. Endocrinol. Metab. 2014 Oct 24 [PMID: 25343232] (IP, Human)

Niemi H, Honkonen K, Korpisalo P et al. HIF-1a and HIF-2a induce angiogenesis and improve muscle energy recovery. Eur J Clin. Invest. 2014 Oct 01 [PMID: 25208310]

Xiao W, Shinohara M, Komori K et al. The importance of physiological oxygen concentrations in the sandwich cultures of rat hepatocytes on gas-permeable membranes. Biotechnol. Prog. 2014 Jul 30 [PMID: 25078970]

Motzer RJ, Hutson TE, Hudes GR et al. Investigation of novel circulating proteins, germ line single-nucleotide polymorphisms, and molecular tumor markers as potential efficacy biomarkers of first-line sunitinib therapy for advanced renal cell carcinoma. Cancer Chemother. Pharmacol. 2014 Aug 07 [PMID: 25100134] (IHC-P, Human)

Details:
Mouse monoclonal HIF-1 alpha antibody used for IHC on formalin-fixed paraffin-embedded (FFPE) blocks or unstained slides from tumor specimens.

Kaira K, Murakami H, Endo M et al. Biological Correlation of 18F-FDG Uptake on PET in Pulmonary Neuroendocrine Tumors. Anticancer Res. 2013 Oct [PMID: 24122985]

Chaux A, Albadine R, Schultz L et al. Dysregulation of the mammalian target of rapamycin pathway in chromophobe renal cell carcinomas. Hum Pathol. 2013 Aug 15 [PMID: 23953228] (IHC-P, Human)

Zheng X, Ruas JL, Cao R et al. Cell-type-specific regulation of degradation of hypoxia-inducible factor 1a: Role of subcellular compartmentalization. Mol Cell Biol 2006 Jun [PMID: 16738327] (IP, WB, Rabbit)

Anderson KM, Tsui P, Guinan P et al. The proliferative response of hela cells to 2-deoxy-D-glucose under hypoxic or anoxic conditions: an analogue for studying some properties of in vivo solid cancers. Anticancer Res 2006 Nov-Dec [PMID: 17201127] (IHC, WB, Human)

Namas RA, Metukuri MR, Dhupar R et al. Hypoxia-induced overexpression of BNIP3 is not dependent on hypoxia-inducible factor 1? in mouse hepatocytes. Shock. 2011 Aug [PMID: 21558981] (WB, Mouse)

Salem AF, Howell A, Sartini M. Downregulation of stromal BRCA1 drives breast cancer tumor growth via upregulation of HIF-1 alpha, autophagy and ketone body production. Cell Cycle. 2012 Nov [PMID: 23047605] (WB, Human)

Diensthuber M, Potinius M, Rodt T et al. Expression of bcl-2 is associated with microvessel density in olfactory neuroblastoma. J Neurooncol 2008 Sep [PMID: 18431543] (IHC-P)

More publications at http://www.novusbio.com/NB100-123

Procedures

Western Blot protocol specific for HIF-1 alpha Antibody (NB100-123)
Protocol specific for NB100-123 Monoclonal Anti-HIF-1 alpha

Western Blot Protocol

1. Resolve aliquots (25-30 ug) of induced nuclear protein extracts on a 4-20% Tris-HCl gel.
2. Transfer proteins to nitrocellulose membrane in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membrane for 1 hour with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4. Incubate membrane overnight at 4C in NB 100-123 diluted in 1X western wash/5% NFDM.
5. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
6. Incubate membrane with HRP conjugated anti-mouse IgG for 1 hour (RT) in 1X western wash/5% NFDM.
7. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
8. Drain membrane and place on saran wrap.
9. Using Amersham ECL Kit, mix equal volumes of two reagents. Pour over membrane (protein side facing up). Let solution sit on membrane for 15-20 seconds.
10. Drain membrane and place on new saran wrap.
11. Wrap up membrane and expose to film.
12. Develop accordingly.

10X Western wash: 24.2 g Tris, 80g NaCl, Tween-20 to 1%, pH 7.6 and QS to 4L.
Stripping buffer: 100 mM BME 2% SDS 62.5 mM Tris (pH 6.7)
To strip membrane: Incubate membrane in stripping buffer for 30 minutes at 56C. Wash membrane for 15 minutes with several change of 1X western wash.

Notes: If hypoxia treatment is not hypoxic enough (less than 2% oxygen to get an induction), signal will be absent. Also, if the harvest time is too slow or there are not enough protease inhibitors, etc., the induced protein will be rapidly lost as HIF-1alpha has a very short half-life.
Nuclear Extract Preparation Reference: Wang and Semenza. Purification and Characterization of Hypoxia-Inducible Factor. Journal of Biological Chemistry. 270(3): 1230-1237, 1995.

**This antibody has demonstrated varying results in Western blot applications. Product NB100-105 is recommended for most Western blot experiments.

Immunohistochemistry Protocol (NB100-123)
Please see:

Primary Reference: Zhong, H., et al. Overexpression of Hypoxia-inducible Factor 1alpha in Common Human Cancers and Their Metastases. Cancer Research. 59: 5830-5835, 1999.

Immunocytochemistry/Immunofluorescence Protocol for HIF-1 alpha Antibody (NB100-123)

1. Fix cells in 3% Paraformaldehyde in PBS for 15 minutes at room temperature, gently rocking.
2. Rinse cells 3 times for 5 minutes in PBS.
3. Block and permeabilize cells in 2% non-fat dry milk (NFDM) dissolved in PBS with 0.1% TX-100 overnight at 4C (covered to prevent evaporation).
4. Rinse cells 3 times for 5 minutes in PBS.
5. Dilute NB100-123 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
6. Place cover slip upside down on a 50 ul drop of diluted antibody on parafilm, in humidity box.
7. Incubate for 1 hour at 37C.
8. Flip slips right side up in wells and rinse 3 times for 5 minutes each, in PBS.
9. In an amber microfuge tube, dilute secondary antibody (Cy3 anti-ms IgG) 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
10. Place 800 ul of diluted secondary antibody in each well and make sure the fluid film covers over the cells on the slip. Alternatively, secondary antibody can be applied in the same manner as the primary (slip upside-down on drop of secondary that has been placed on a sheet of parafilm that is inside of a humidity box).
11. Incubate for 1 hour at 37C, in the dark.
12. Rinse cells at room temperature 4 times for 15 minutes each, in PBS, gently rocking.
13. Mount on frosted slides with AquaPoly Mount (Polysciences).
14. Refrigerate flat and covered.

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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.

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