Product Datasheet

HIF-1 alpha Antibody (ESEE122)
NB100-131

Unit Size: 0.1 ml

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

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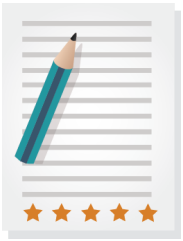
Updated 12/20/2023 v.20.1



NB100-131

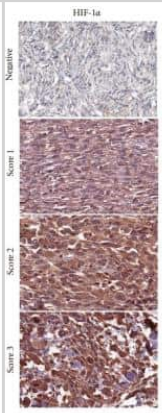
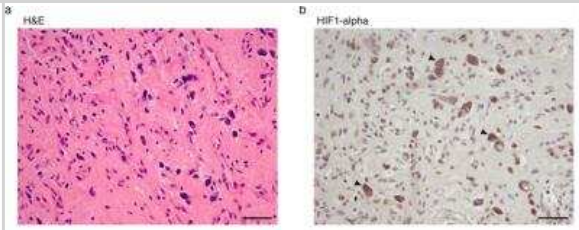
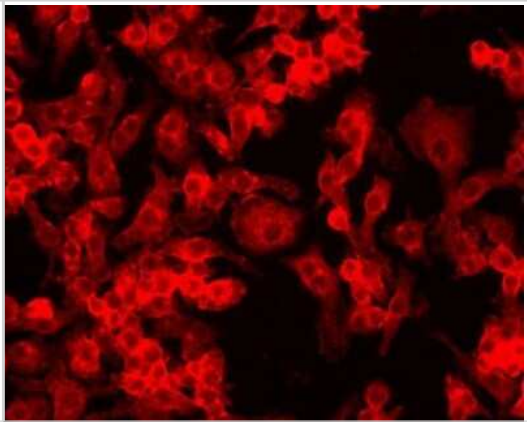
HIF-1 alpha Antibody (ESEE122)

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	Monoclonal
Clone	ESEE122
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	93 kDa

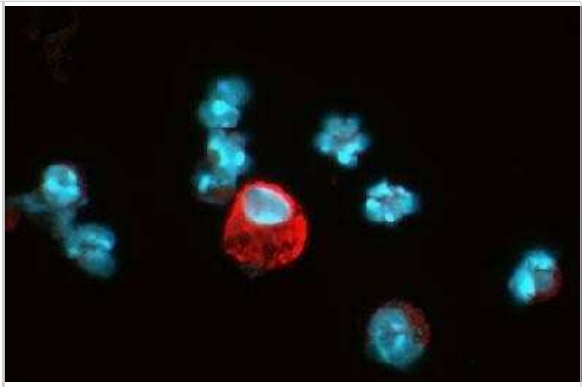
Product Description	
Host	Mouse
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Bovine, Canine
Reactivity Notes	Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.
Immunogen	This HIF-1 alpha Antibody (ESEE122) was developed against Human HIF-1 alpha, corresponding to amino acids 329 - 530 [Uniprot# Q16665].

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:1000, Simple Western 1:2000, Flow Cytometry reported in scientific literature (Gestier S. et al), Immunohistochemistry 1:100-1:5000, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10-1:500. Use reported in scientific literature (PMID 26757928 Fig1G), Immunohistochemistry-Paraffin 1:100-1:5000, Immunohistochemistry-Frozen 1:100-1:5000, Immunoblotting
Application Notes	Variable results have been obtained in Western blot. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

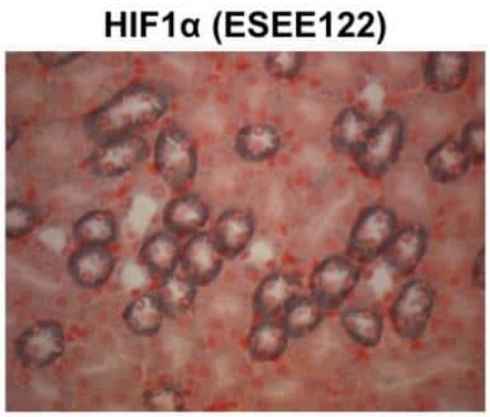
Images

Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical analysis in non-GIST STS representing negative, and score 1-3 of Carbonic Anhydrase IX/CA9, GLUT-1, HIF-1 alpha, and HIF-2 alpha/EPAS1. non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, Carbonic Anhydrase IX/CA9: carbonic anhydrase IX, GLUT-1: glucose transporter-1, and HIF-1/2alpha: hypoxia induced factor 1/2alpha. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/sarcoma/2012/541650/), licensed under a CC-BY license.	
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. Representative images of sample SH-0622 acquired at 400x of (a) H + E and IHC for (b) HIF1-alpha reveal heterogenous cell types in this tumor characterized by a rich vascular network. Arrowheads indicate that the stromal cells demonstrate increased cytoplasmic staining for HIF1-alpha and VEGF, whereas the double arrowheads highlight PDGFR-beta protein restricted to vascular endothelium. Scale bar is 25 um. Image collected and cropped by CiteAb from the following publication (https://www.actaneurocomms.org/content/2/1/167), licensed under a CC-BY license.	
Simple Western: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Image 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.	
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line, using NB100-131. Photos courtesy of Susan Alexander and Hattie Gresham, PhD.	

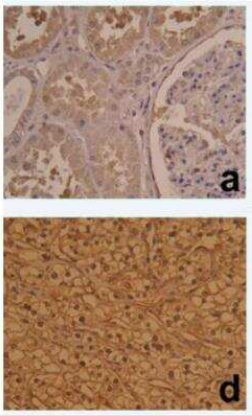
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with S. aureus. 100X magnification. Blue: DAPI nuclear staining.



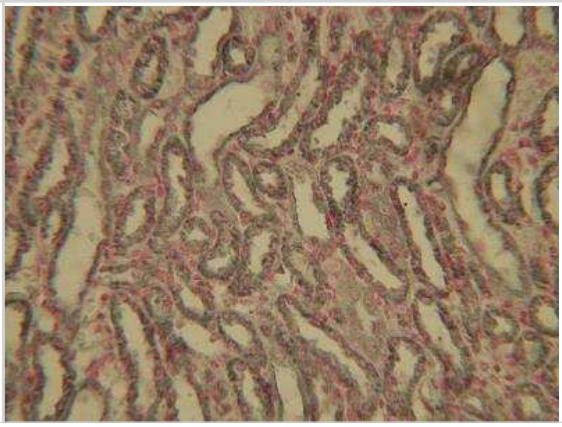
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE mouse kidney tissue section using HIF-1 alpha antibody clone ESEE122 at 1ug/mL concentration. The detection was performed using X-cell plus universal HRP polymer detection system with Vector SG chromagen substrate. Image courtesy of a product review by Steven Grover.



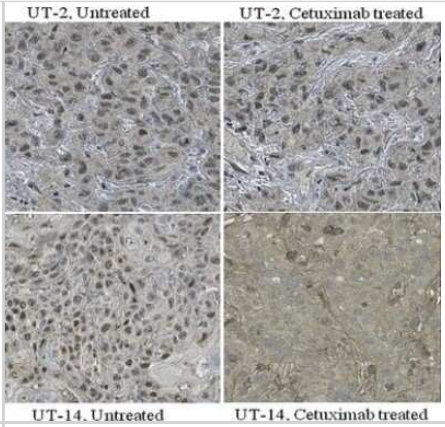
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical staining of HIF-1 alpha in normal renal tissue (A) and clear cell renal cell carcinoma (CCRCC) (D). A homogeneous cytoplasmic staining of tubular cells and weak staining in glomerules was observed with HIF-1 alpha (A). In CCRCC, HIF-1 alpha immmunoreactivity was nuclear and/or cytoplasmic (D), while it was perimembranous and/or diffuse cytoplasmic for VEGF-A and VEFG-C (E and F). (magnification x200). Image collected and cropped by CiteAb from the following publication (https://www.jeccr.com/content/28/1/40), licensed under a CC-BY license.



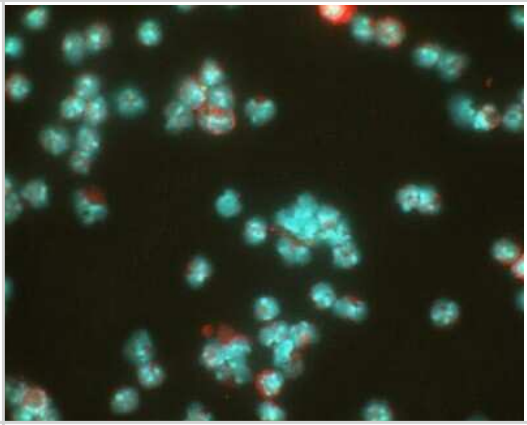
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of HIF-1 alpha in paraffin-embedded mouse kidney tissue section using anti-HIF-1 alpha antibody. Image from verified customer review.



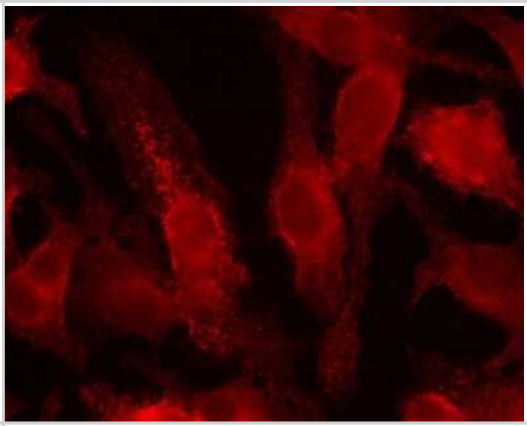
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Nuclear HIF-1 alpha protein expression. Xenografts were established in female nude mice (BALB c[nu/nu]) by subcutaneous injection of head and neck squamous cell carcinoma cell lines UT-SCC-2 (UT-2) and UT-SCC-14 (UT-14). Cetuximab (1 mg/injection) or PBS was administered by intraperitoneal injection at day 10, 14, and 17. A tissue microarray was constructed from tumours harvested at day 21, and the expression of nuclear HIF-1 alpha was evaluated by immunohistochemistry (IHC) in untreated controls and cetuximab-treated tumour specimens. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/28756482/) licensed under a CC-BY license.



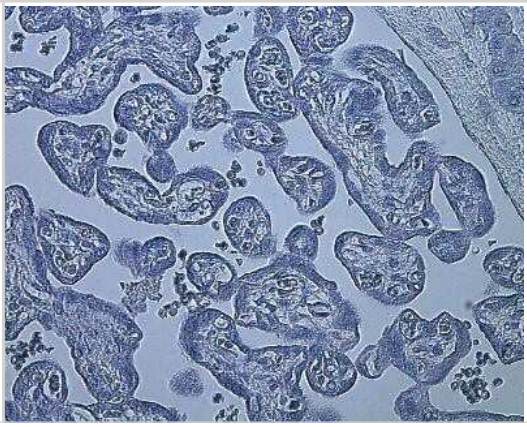
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with S. aureus, using NB100-131. Blue: DAPI nuclear staining. Image courtesy of Susan Alexander and Hattie Gresham, PhD.

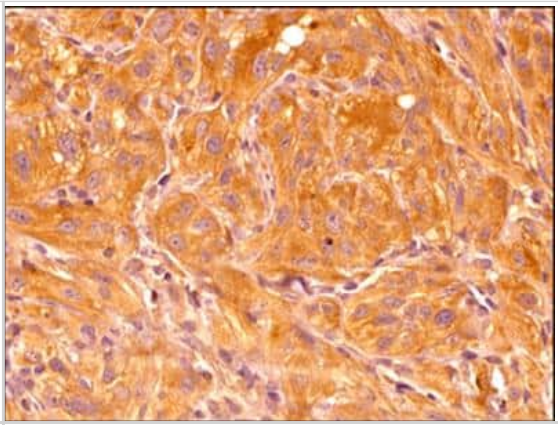
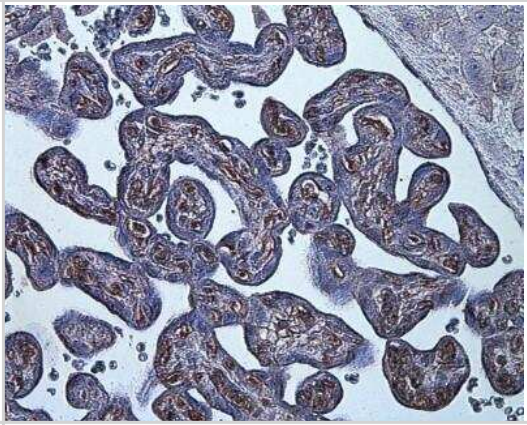
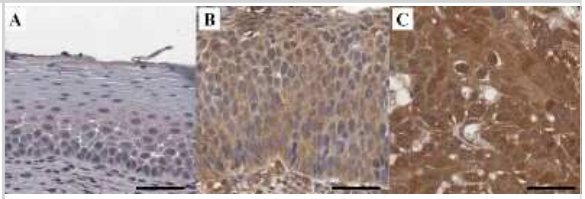
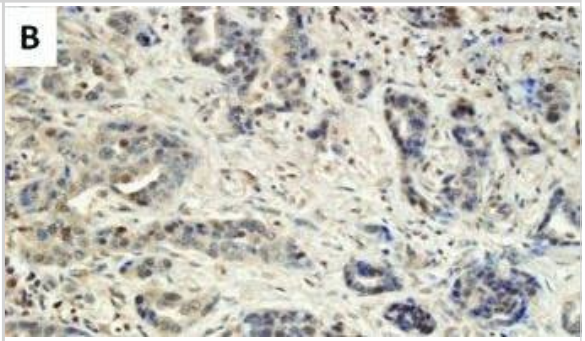


Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line. 100X magnification. Image courtesy of Susan Alexander and Hattie Gresham, PhD.

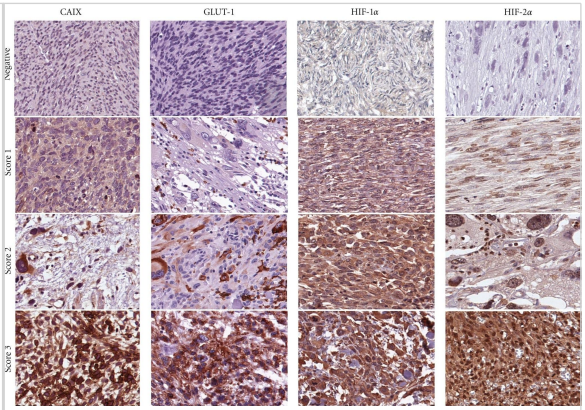


Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Negative control stain of human placenta (from sea level) using mouse IgG at 1:100. 4uM paraffin-embedded section.

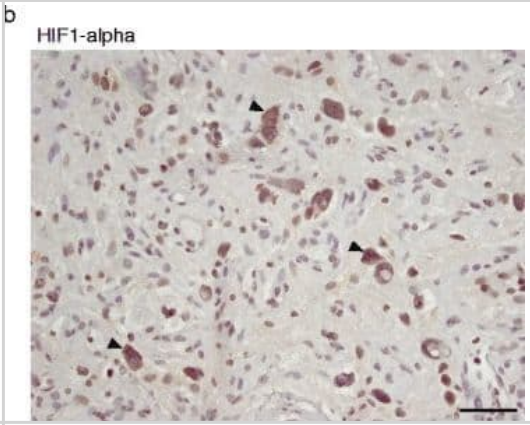


Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE tissue section of human renal cancer xenograft using HIF-1 alpha antibody (NB100-131 Lot 83115) at 1:200 dilution. The antibody generated a strong cytoplasmic staining mainly in the cancer cells. Only a fraction of cells depicted nuclear staining, while weak to negligible positivity was seen in the tumor stromal cells.	
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - HIF-1 alpha staining in hypoxia-induced human placenta.	
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Representative immunohistochemical expression for HIF-1alpha, c-Met, CA9 and GLUT1. HIF-1alpha is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), and strong staining intensity in squamous cell carcinoma (C). Scale bar: 50 um. Image collected and cropped by CiteAb from the following publication (https://www.translational-medicine.com/content/11/1/185), licensed under a CC-BY license.	
Positive immunohistochemical staining for (A) VEGF, (B) HIF-1α, (C) Dll4 (tumor cells), (D) Dll4 (endothelial cells), and (E) CD31 (for microvessel counting, ×200 magnification).	

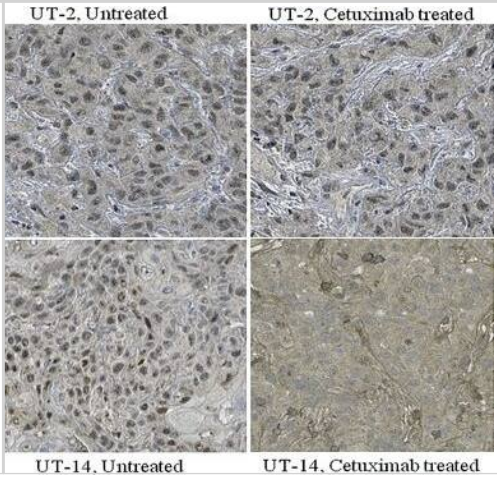
Immunohistochemical analysis in non-GIST STS representing negative, and score 1–3 of CAIX, GLUT-1, HIF-1α, and HIF-2α. non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, CAIX: carbonic anhydrase IX, GLUT-1: glucose transporter-1, and HIF-1/2α: hypoxia induced factor 1/2α.



Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. "Representative images of sample SH-0622 acquired at 400x of (a) H + E and IHC for (b) HIF1-alpha, (c) VEGF, and (d) PDGFR-beta reveal heterogenous cell types in this tumor characterized by a rich vascular network. Arrowheads indicate that the stromal cells demonstrate increased cytoplasmic staining for HIF1-alpha and VEGF, whereas the double arrowheads highlight PDGFR-beta protein restricted to vascular endothelium. Scale bar is 25 um. Image collected and cropped by CiteAb from the following publication (https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-014-0167-x), licensed under a CC-BY licence.

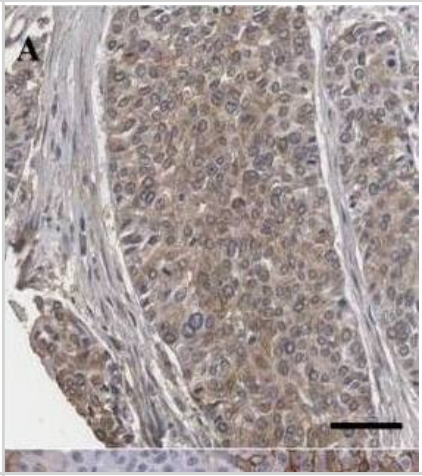


Nuclear HIF-1alpha protein expression. Xenografts were established in female nude mice (BALB c[nu/nu]) by subcutaneous injection of head and neck squamous cell carcinoma cell lines UT-SCC-2 (UT-2) and UT-SCC-14 (UT-14). Cetuximab (1 mg/injection) or PBS was administered by intraperitoneal injection at day 10, 14, and 17. A tissue microarray was constructed from tumours harvested at day 21, and the expression of nuclear HIF-1alpha was evaluated by immunohistochemistry (IHC) in untreated controls and cetuximab-treated tumour specimens. Bar graphs showing the IHC staining score for HIF-1alpha. One way ANOVA and post hoc Tukey's HSD test were used to test differences between treated and untreated groups (*p <= 0.05), n = 10-14 Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28756482), licensed under a CC-BY licence.

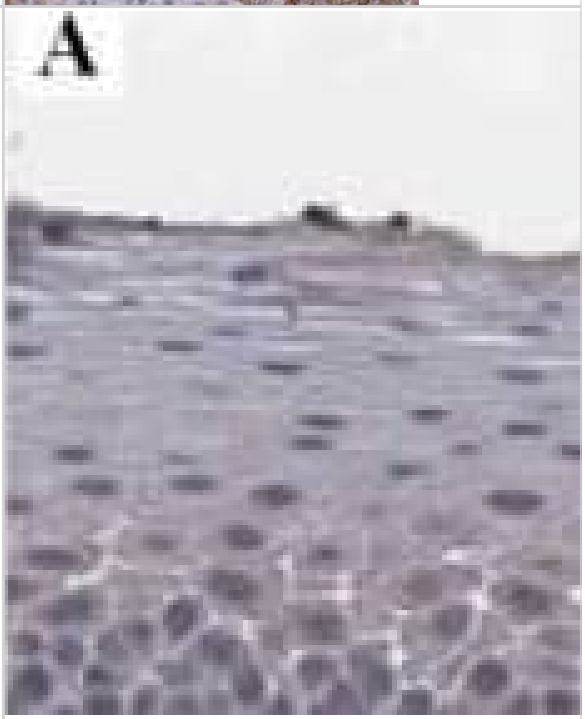


TAC increases Dec1 expression. (A) The circadian expression of clock genes in WT mice treated with TAC (red dotted line) and sham treatment (black line). The mRNA levels of Dec1, Dec2, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 (Bmal1), and period 2 (Per2) were analyzed by real-time PCR. Each right graph shows average of total mRNA expressions from zeitgeber time 2 (ZT2) to ZT22 in sham and TAC mice. The circadian expression of clock genes was assessed by analyzing one-way ANOVA. Multiple comparisons between sham and TAC groups were analyzed by two-way ANOVA with Tukey–Kramer post hoc test. Comparison of two groups was analyzed by a two-tailed Student's t-test. The number of mice was four or five mice per group per time point. Data are the means ± SEM. ** $p < 0.01$; NS: not significant; ZT: zeitgeber time with light on at 8:00 a.m. (ZT0) and light off at 8:00 p.m. (ZT12). (B) Immunohistochemical detection of Dec1 in myocardial and stromal cells. Representative images of one WT heart treated with TAC and sham at four weeks. The black square shows representative large images, magnification 400×. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31597354), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

BRD4770-induced activation of the FA pathway may occur via inhibition of the PRC2 complex(A) U2OS cells were incubated in the absence (NT) or presence of BRD4770, DZNep, UNC0646, and DZNep and UNC0646 combined (4 μM each) for 24 h. Whole-cell lysates were prepared and immunoblotted with anti-FANCD2, anti-FANCI, anti-EZH2, and anti-α-Tubulin antibodies. (B) U2OS cells were incubated in the absence or presence of 2, 5, and 10 μM BRD4770 for 24, 48, or 72 h. Whole-cell lysates were prepared and immunoblotted with anti-FANCD2, anti-FANCI, anti-CHK1 pS345, anti-EZH2, anti-H3K27me3, and anti-α-Tubulin antibodies. L:S Ratio, ratio of monoubiquitinated to nonubiquitinated FANCI; or FANCD2 RBI, relative band intensity. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29100324), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Rapamycin increases autophagy in brains of PDAPP mice.a, f and h, representative immunoblots of hippocampal lysates from control- and rapamycin-treated transgenic PDAPP mice and non-transgenic littermate controls. b, g and i, quantitative analyses. a and b, LC3-II levels are decreased in hippocampi of rapamycin-treated transgenic PDAPP mice (*, P=0.0009), but not in hippocampi of rapamycin-treated non-transgenic littermates. c and d, representative epifluorescent (c, 200×) and higher-magnification confocal (d, 600×) images of hippocampal CA1 (e, green box, region of epifluorescent images; blue box, region of confocal images) in control- and rapamycin-fed transgenic PDAPP mice stained with an anti-LC3 antibody. An increase in LC3-immunoreactive puncta was observed in CA1 projections of transgenic PDAPP mice following rapamycin administration. f and g, levels of the autophagic substrate p62SQSTM are decreased (*, P=0.0015) in hippocampi of rapamycin-treated PDAPP transgenic mice. f, representative Western blots; g, quantitative analyses of p62SQSTM levels. h and i, Levels of phosphorylated (activated) p70 were decreased in brains of rapamycin-treated PDAPP and non-transgenic mice (*, P=0.001 and P=0.04 respectively). Significance of differences between group means were determined using two-tailed unpaired Student's t test. Data are means ± SEM. Image collected and cropped by CiteAb from the following open publication (https://dx.plos.org/10.1371/journal.pone.0009979), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Kim JH, Lee ES, Yun J et al. Calsequestrin 2 overexpression in breast cancer increases tumorigenesis and metastasis by modulating the tumor microenvironment Molecular Oncology 2022-01-01 [PMID: 34743414]

Yoo JY, Kim HB, Lee YJ et al. Neuregulin-1 reverses anxiety-like behavior and social behavior deficits induced by unilateral micro-injection of CoCl2 into the ventral hippocampus (vHPC) Neurobiology of disease 2022-12-30 [PMID: 36592864] (WB, ICC/IF, IHC-Fr, Mouse)

Details:
Dilution used in IHC-Fr and ICC/IF 1:100. Dilution used in WB 1:1000

Wang H, Tang C, Dang Z et al. Clinicopathological characteristics of high-altitude polycythemia-related kidney disease in Tibetan inhabitants. Kidney International 2022-05-01 [PMID: 35513124] (IF/IHC, Human)

Zhao L, Han Q, Zhou L et al. Addition of glomerular lesion severity improves the value of anemia status for the prediction of renal outcomes in Chinese patients with type 2 diabetes Renal failure 2022-12-01 [PMID: 35188068] (IF/IHC, Human)

Schuman ML, Diaz LSP, Aisicovich M et al. Cardiac thyrotropin-releasing hormone (TRH) inhibition improves ventricular function and reduces hypertrophy and fibrosis after myocardial infarction in rats Journal of cardiac failure 2021-04-15 [PMID: 33865967]

Ebright RY, Zachariah MA, Micalizzi DS et al. HIF1A signaling selectively supports proliferation of breast cancer in the brain Nature communications 2020-12-09 [PMID: 33298946] (IHC-P, Human, Mouse)

Zhao L, Wang X, Wang T et al. Associations Between High-Altitude Residence and End-Stage Kidney Disease in Chinese Patients with Type 2 Diabetes High Alt Med Biol 2020-11-12 [PMID: 33185478] (IHC-P, Human)

Kim HB, Yoo JY, Yoo SY et al. Neuregulin-1 inhibits CoCl2-induced upregulation of excitatory amino acid carrier 1 expression and oxidative stress in SH-SY5Y cells and the hippocampus of mice Mol Brain 2020-11-13 [PMID: 33187547]

Scarlato M, Previtali S C et al. Polyneuropathy in POEMS syndrome: role of angiogenic factors in the pathogenesis. Brain 2005-01-08 [PMID: 15975949] (ICC/IF, Human)

Minervini A, Di Cristofano C et al. Hypoxia-inducible factor 1alpha expression in renal cell carcinoma analyzed by tissue microarray Eur Urol 2007-01-05 [PMID: 17239525] (IF/IHC, Human)

Gibbon N Transcapsular adenomectomy (Millin): a comparative study, extraperitoneal laparoscopy versus open surgery. Eur Urol 2007-01-05 [PMID: 17239526] (IF/IHC, Human)

Maeda A, Nakata M et al. Influence of vascular endothelial growth factor single nucleotide polymorphisms on non-small cell lung cancer tumor angiogenesis. Oncol Rep 2013-01-01 [PMID: 23064377] (IF/IHC, Human)

More publications at http://www.novusbio.com/NB100-131

Procedures

Immunohistochemistry protocol for HIF-1 alpha Antibody (NB100-131)

Immunohistochemistry Procedures
Paraffin Sections

1.Prior to performing the IPOX (immunoperoxidase) experiment, dewax the paraffin sections by baking them at 60 degrees C for 30 minutes and then putting them through citroclear [Citroclear is a mounting agent (chemical name Limonene, also known as Histoclear, Bioclear)].
2.Hydrate the sections through the following series:
A.3 X 5 minutes xylenes
B.3 X 5 minutes 100% Etoh
C.2 minutes 95% Etoh
D.2 minutes 70% Etoh
E.1 minute 50% Etoh
F.1 minute ddH2O
G.1 minute TBS

1.Block endogenous peroxidase with 0.5% hydrogen peroxide in water, for 30 minutes.
2. Antigen unmasking is performed by incubating at 60 degrees C for 16 hours, in 50mmol/L Tris and 0.2 mmol/L EDTA (pH 9.0), using a covered water bath.
1.Rinse slides with PBS and then incubate with PBS containing 0.2% Triton X-100 for 10 minutes.
2.Rinse slides with PBS.
3. Incubate sections with 1:8000 dilution of anti-HIF-1 alpha (NB100-131) for 90 minutes at room temperature (RT).
4. Incubate sections in secondary HRP-conjugated goat anti-mouse serum for 30 minutes at RT.
5. Incubate sections in tertiary HRP-conjugated rabbit anti-goat serum for 30 minutes at RT.
6. Develop the peroxidase reaction using diaminobenzidine.
7. Wash slide and mount in aqueous mountant.
Substitution of the primary antibody with PBS can be used as a negative control.
1. Deparaffinize to water:Xylene #1-10 dipsXylene #2-10 dips 100%EtOH #1-10 dips 100%EtOH #2-10 dips 95% EtOH-10 dips 70%EtOH-10 dips diH2O-2 changes
2. Rinse in PBS for two minutes.
3. Quench slides is MeOH/H2O2 for 5-10 minutes (1 part 30% H2O2/36 parts 70% MeOH; 8 mls H2O2/288 mls 70% MeOH).
4. Unmask antigens by boiling for 3 minutes in 0.01 M Citrate Buffer, pH 5.5. 47.2gr Sodium Citrate 8.3gr Citric Acid pH to 5.5 qs to 0.5 L dH2O
5. Rinse in PBS.
6. Apply 2 drops blocking solution (10% non-immune normal goat serum, Zymed Labs, Cat # 50-197). Incubate for 10 minutes in humidity chamber
7. Incubate for 10 minutes in humidity chamber.
8. Do not rinse.
9. Incubate in mAb HIF-1 alpha (cat# NB 100-131), diluted 1:250 in PBS (10ul /2.5mls) overnight at 4 degrees C, in humidity chamber.
10. Rinse in PBS.
11. Incubate in 2 drops Biotinylated Secondary Antibody for 10 minutes in humidity chamber.
12. Rinse in PBS.
13. Incubate in 2 drops Enzyme Conjugate solution (HRP-Streptavidin) for 10 minutes in humidity chamber.
14. Rinse in PBS.
15. Incubate in 2 drops Substrate-Chromatogen solution AEC solution, (AEC Single Solution, Zymed Labs, Cat# 00-1111) for 5-10 minutes in humidity chamber.
16. Rinse well in dH20.
17. Counterstain with hematoxylin for 1 minute.
18. Rinse well in tap water until it runs clear.
19. Mount coverslip with water soluble mounting media. Do not dehydrate. (Alcohols will remove the AEC color).

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