

# Product Datasheet

## HIF-1 alpha Antibody (ESEE122) - BSA Free NB100-131

Unit Size: 0.1 ml

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

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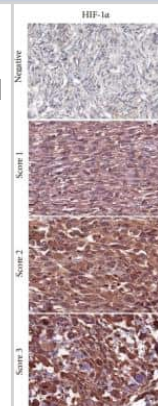
**NB100-131**

HIF-1 alpha Antibody (ESEE122) - BSA Free

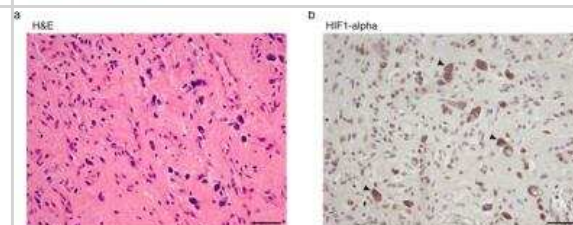
<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	ESEE122
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG1
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	93 kDa
<b>Product Description</b>	
<b>Host</b>	Mouse
<b>Gene ID</b>	3091
<b>Gene Symbol</b>	HIF1A
<b>Species</b>	Human, Mouse, Rat, Bovine, Canine
<b>Reactivity Notes</b>	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.
<b>Immunogen</b>	This HIF-1 alpha Antibody (ESEE122) was developed against Human HIF-1 alpha, corresponding to amino acids 329 - 530 [Uniprot# Q16665].
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
<b>Recommended Dilutions</b>	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
<b>Application Notes</b>	Variable results have been obtained in Western blot.  In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in Hypoxic HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:2000. 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.



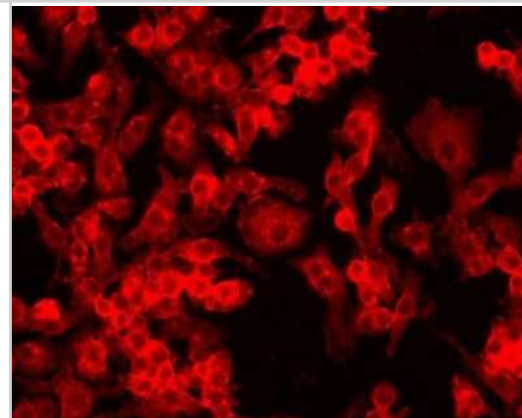
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. Representative images of sample SH-0622 acquired at 400x of (a) H + E and IHC for (b) HIF1-alpha reveal heterogenous cell types in this 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.



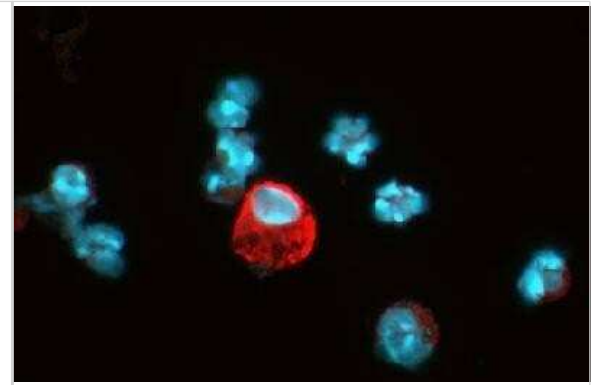
Simple Western: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Image shows a specific band for HIF-1 alpha in 0.5 mg/mL of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



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.

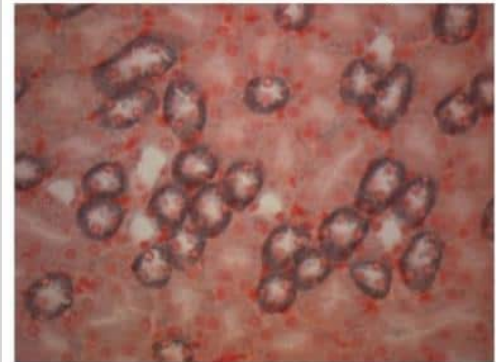


Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with *S. aureus*. 100X magnification. Blue: DAPI nuclear staining.

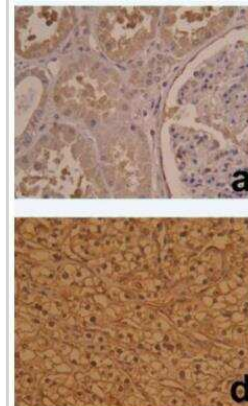


Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE mouse kidney tissue section using HIF-1 alpha antibody clone ESEE122 at 1ug/mL concentration. The detection was performed using X-cell plus universal HRP polymer detection system with Vector SG chromagen substrate.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

### HIF1 $\alpha$ (ESEE122)



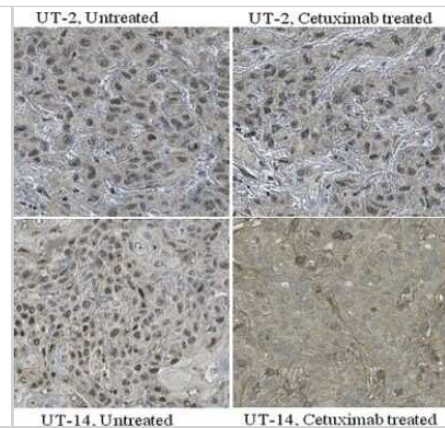
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical staining of HIF-1 alpha in normal renal tissue (A) and clear cell renal cell carcinoma (CCRCC) (D). A homogeneous cytoplasmic staining of tubular cells and weak staining in glomerules was observed with HIF-1 alpha (A). In CCRCC, HIF-1 alpha immunoreactivity was nuclear and/or cytoplasmic (D), while it was perimembranous and/or diffuse cytoplasmic for VEGF-A and VEGF-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.



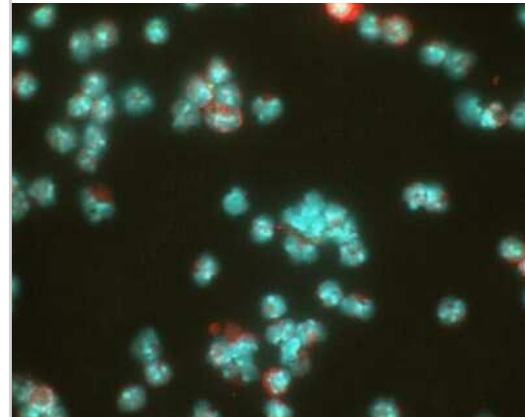
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of HIF-1 alpha in paraffin-embedded mouse kidney tissue section using anti-HIF-1 alpha antibody. Image from verified customer review.



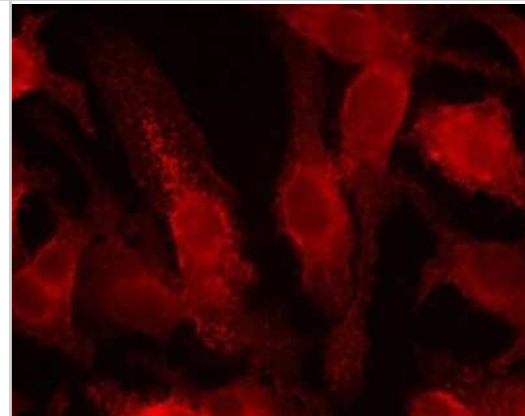
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Nuclear HIF-1 alpha protein expression. Xenografts were established in female nude mice (BALB c[*nu/nu*]) by subcutaneous injection of head and neck squamous cell carcinoma cell lines UT-SCC-2 (UT-2) and UT-SCC-14 (UT-14). Cetuximab (1 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/](https://pubmed.ncbi.nlm.nih.gov/28756482/)) licensed under a CC-BY license.



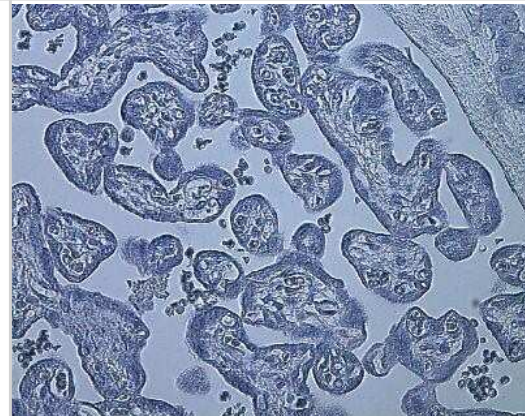
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with *S. aureus*, using NB100-131. Blue: DAPI nuclear staining. Image courtesy of Susan Alexander and Hattie Gresham,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



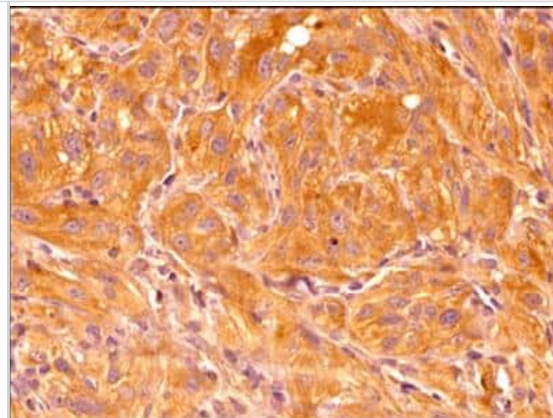
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line. 100X magnification. Image courtesy of Susan Alexander and Hattie Gresham, PhD.



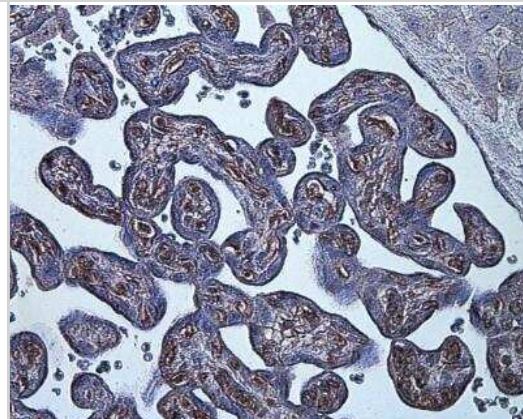
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Negative control stain of human placenta (from sea level) using mouse IgG at 1:100. 4uM paraffin-embedded section.



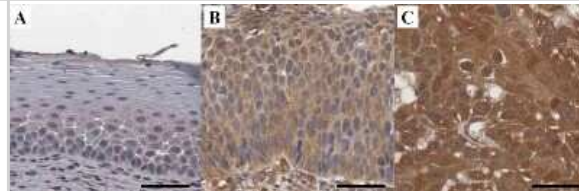
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE tissue section of human renal cancer xenograft using HIF-1 alpha antibody (NB100-131 Lot 83115) at 1:200 dilution. The antibody generated a strong cytoplasmic staining mainly in the cancer cells. Only a fraction of cells depicted nuclear staining, while weak to negligible positivity was seen in the tumor stromal cells.



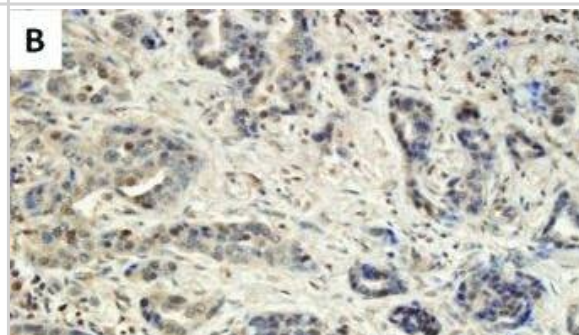
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - HIF-1 alpha staining in hypoxia-induced human placenta.



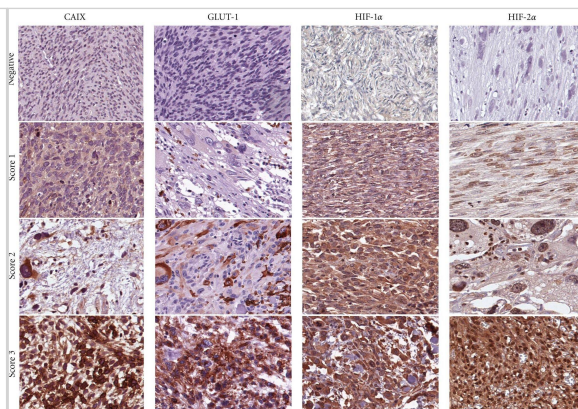
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Representative immunohistochemical expression for HIF-1alpha, c-Met, CA9 and GLUT1. HIF-1alpha is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), and strong staining 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.



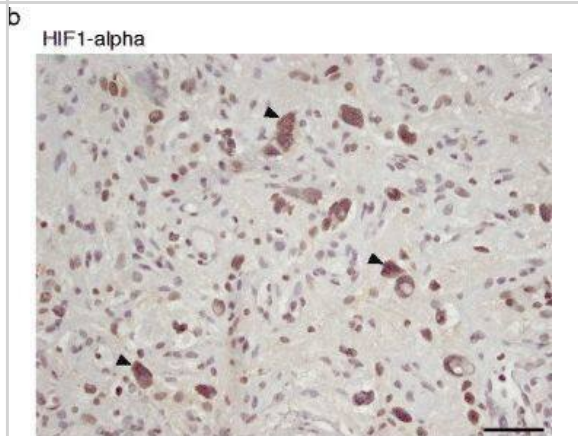
Positive immunohistochemical staining for (A) VEGF, (B) HIF-1 $\alpha$ , (C) DII4 (tumor cells), (D) DII4 (endothelial cells), and (E) CD31 (for microvessel counting,  $\times 200$  magnification).



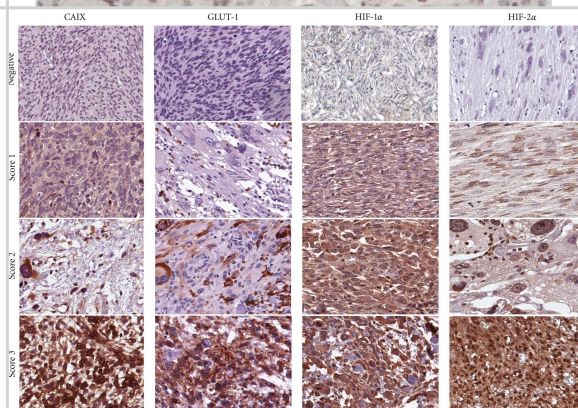
Immunohistochemical analysis in non-GIST STS representing negative, and score 1–3 of CAIX, GLUT-1, HIF-1 $\alpha$ , and HIF-2 $\alpha$ . non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, CAIX: carbonic anhydrase IX, GLUT-1: glucose transporter-1, and HIF-1/2 $\alpha$ : hypoxia induced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



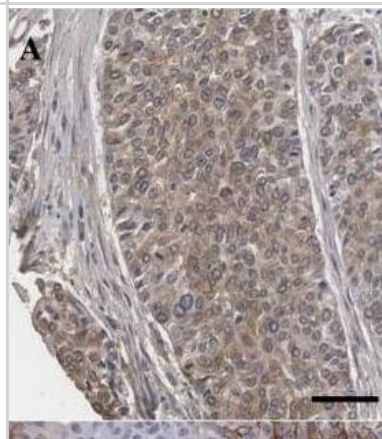
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. “Representative images of sample SH-0622 acquired at 400x of (a) H + E & IHC for (b) HIF1- $\alpha$ , (c) VEGF, & (d) PDGFR- $\beta$  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 & VEGF, whereas the double arrowheads highlight PDGFR-beta protein restricted to vascular endothelium. Scale bar is 25  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-014-0167-x>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



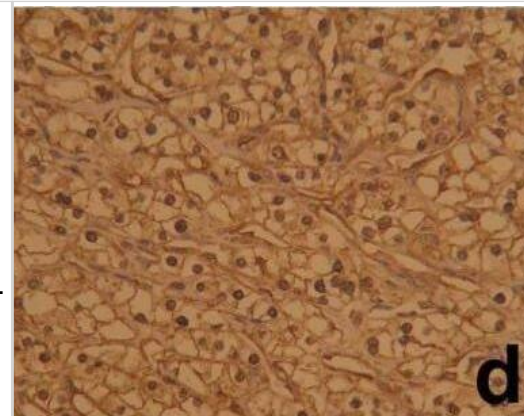
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical analysis in non-GIST STS representing negative, & score 1–3 of CAIX, GLUT-1, HIF-1 $\alpha$ , & HIF-2 $\alpha$ . non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, CAIX: carbonic anhydrase IX, GLUT-1: glucose transporter-1, & HIF-1/2 $\alpha$ : hypoxia induced factor 1/2 $\alpha$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22454562>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



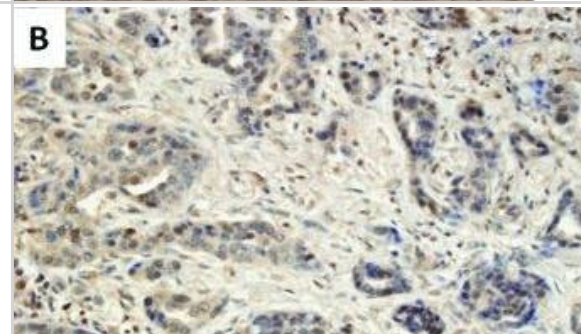
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Digital image analysis of cytoplasmic & membranous staining. Cytoplasmic HIF-1 $\alpha$  staining is shown (A) & automated image analysis utilizing TissueIA recognizes cytoplasmic HIF-1 $\alpha$  staining highlighted in green color (B). CA9 is shown in membranous staining (C) & automated image analysis determines membranous CA9 staining highlighted in green color (D). The output from the algorithm returns a number of quantitative measurements for intensity & percentage of positive staining present. Scale bar: 100  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-11-185>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



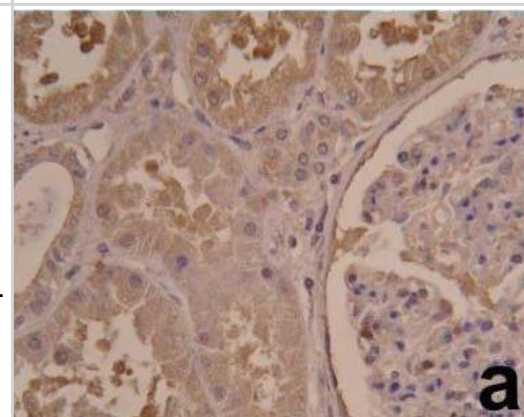
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical staining of HIF-1 $\alpha$ , VEGF-A & VEGF-C in normal renal tissue (A-C) & clear cell renal cell carcinoma (CCRCC) (D-F). A homogeneous cytoplasmic staining of tubular cells & weak staining in glomerules was observed with HIF-1 $\alpha$  (A), while VEGF-A & VEGF-C were positive in tubular cells, glomerular mesangium & interstitial macrophages (B & C). In CCRCC, HIF-1 $\alpha$  immunoreactivity was nuclear and/or cytoplasmic (D), while it was perimembranous and/or diffuse cytoplasmic for VEGF-A & VEGF-C (E & F). (magnification  $\times 200$ ). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/19302703>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



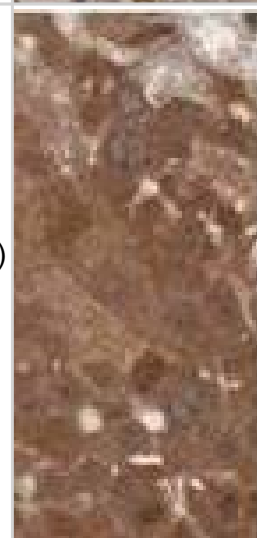
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Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical staining of HIF-1 $\alpha$ , VEGF-A & VEGF-C in normal renal tissue (A-C) & clear cell renal cell carcinoma (CCRCC) (D-F). A homogeneous cytoplasmic staining of tubular cells & weak staining in glomerules was observed with HIF-1 $\alpha$  (A), while VEGF-A & VEGF-C were positive in tubular cells, glomerular mesangium & interstitial macrophages (B & C). In CCRCC, HIF-1 $\alpha$  immunoreactivity was nuclear and/or cytoplasmic (D), while it was perimembranous and/or diffuse cytoplasmic for VEGF-A & VEGF-C (E & F). (magnification  $\times 200$ ). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/19302703>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

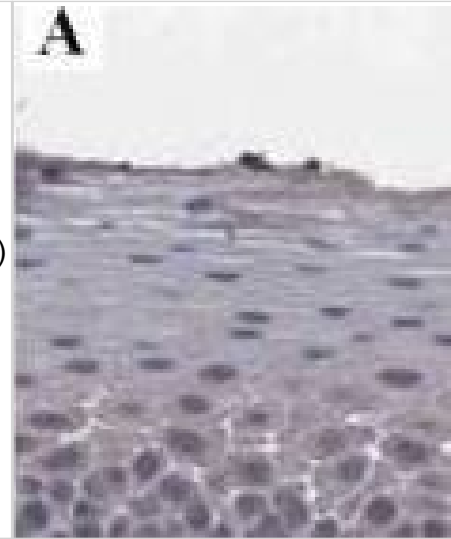


Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Representative immunohistochemical expression for HIF-1 $\alpha$ , c-Met, CA9 & GLUT1. HIF-1 $\alpha$  is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), & strong staining intensity in squamous cell carcinoma (C). c-Met (D-F), CA9 (G, H) & GLUT1 (I) shows cell membranous staining. Representative c-Met expression in cervical samples shown with no staining in normal cervix (D), weak membranous staining intensity in squamous cell carcinoma (E) & strong intensity in squamous cell carcinoma (F). CA9 expression showing moderate intensity staining in carcinoma in situ (CIS) (G) & strong staining in adenocarcinoma (H). GLUT1 expression showing strong intensity in squamous cell carcinoma (I). Scale bar: 50  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-11-185>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Representative immunohistochemical expression for HIF-1 $\alpha$ , c-Met, CA9 & GLUT1. HIF-1 $\alpha$  is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), & strong staining intensity in squamous cell carcinoma (C). c-Met (D-F), CA9 (G, H) & GLUT1 (I) shows cell membranous staining. Representative c-Met expression in cervical samples shown with no staining in normal cervix (D), weak membranous staining intensity in squamous cell carcinoma (E) & strong intensity in squamous cell carcinoma (F). CA9 expression showing moderate intensity staining in carcinoma in situ (CIS) (G) & strong staining in adenocarcinoma (H). GLUT1 expression showing strong intensity in squamous cell carcinoma (I). Scale bar: 50  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-11-185>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Ak Ç, Sayar Z, Thibault G, Burlingame EA et Al. Multiplex imaging of localized prostate tumors reveals altered spatial organization of AR-positive cells in the microenvironment *iScience* 2024-09-09 [PMID: 39246442]

Ka NL, Lim GY, Kim SS et al. Type I IFN stimulates IFI16-mediated aromatase expression in adipocytes that promotes E2-dependent growth of ER-positive breast cancer *Cellular and molecular life sciences : CMLS* 2022-05-20 [PMID: 35593921]

Details:  
Fig. 3

Schlotterose, L;Cossais, F;Lucius, R;Hattermann, K; Resveratrol Alleviates the Early Challenges of Implant-Based Drug Delivery in a Human Glial Cell Model *International journal of molecular sciences* 2024-02-08 [PMID: 38396755]

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 CoCl<sub>2</sub> 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 CoCl<sub>2</sub>-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)

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 ddH<sub>2</sub>O

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 dips Xylene #2-10 dips 100%EtOH #1-10 dips 100%EtOH #2-10 dips 95% EtOH-10 dips 70%EtOH-10 dips diH<sub>2</sub>O-2 changes

2. Rinse in PBS for two minutes.

3. Quench slides in MeOH/H<sub>2</sub>O<sub>2</sub> for 5-10 minutes (1 part 30% H<sub>2</sub>O<sub>2</sub>/36 parts 70% MeOH; 8 mls H<sub>2</sub>O<sub>2</sub>/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 dH<sub>2</sub>O

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 dH<sub>2</sub>O.

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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