

Product Datasheet

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

Unit Size: 0.025 ml

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

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NB100-131SS

HIF-1 alpha Antibody (ESEE122) - BSA Free

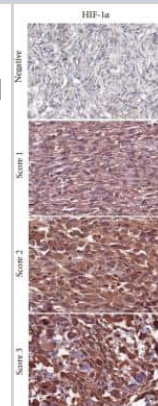
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	ESEE122
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	93 kDa

Product Description	
Description	Novus Biologicals Mouse HIF-1 alpha Antibody (ESEE122) - BSA Free (NB100-131) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF, Simple Western and IP. Anti-HIF-1 alpha Antibody: Cited in 88 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
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].

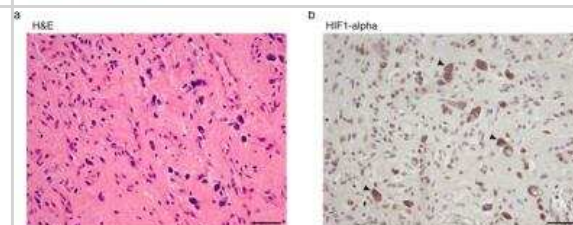
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:1000, Simple 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. See Simple Western Antibody Database for Simple Western validation: Tested in Hypoxic HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:2000. 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.



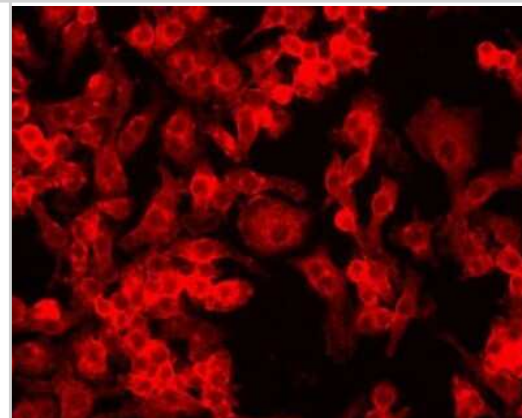
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. Representative images of sample SH-0622 acquired at 400x of (a) H + E and IHC for (b) HIF1-alpha reveal heterogenous cell types in this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



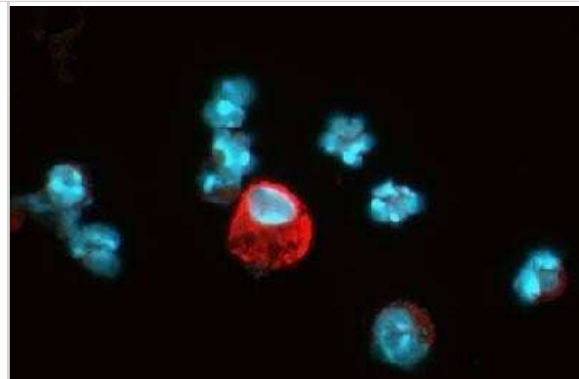
Simple Western: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Image shows a specific band for HIF-1 alpha in 0.5 mg/mL of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



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.

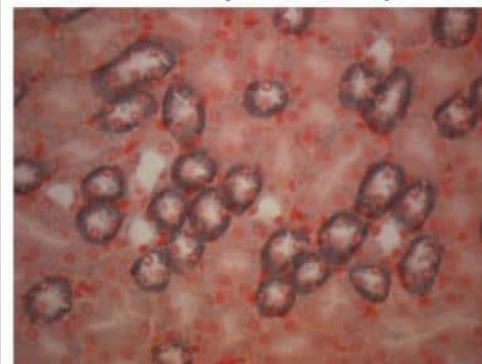


Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with *S. aureus*. 100X magnification. Blue: DAPI nuclear staining.

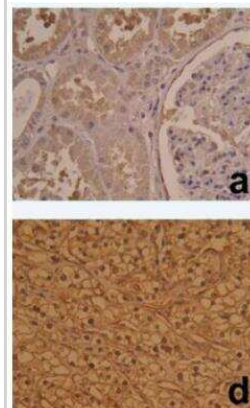


Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE mouse kidney tissue section using HIF-1 alpha antibody clone ESEE122 at 1ug/mL concentration. The detection was performed using X-cell plus universal HRP polymer detection system with Vector SG chromagen substrate.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

HIF1 α (ESEE122)



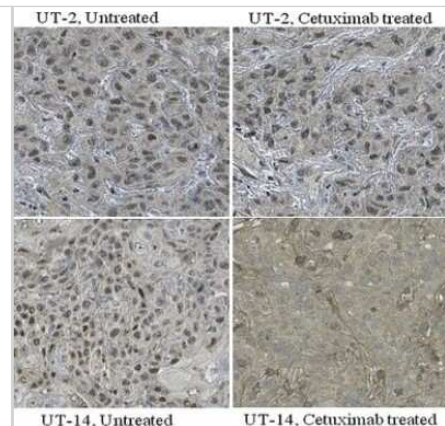
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical staining of HIF-1 alpha in normal renal tissue (A) and clear cell renal cell carcinoma (CCRCC) (D). A homogeneous cytoplasmic staining of tubular cells and weak staining in glomerules was observed with HIF-1 alpha (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.



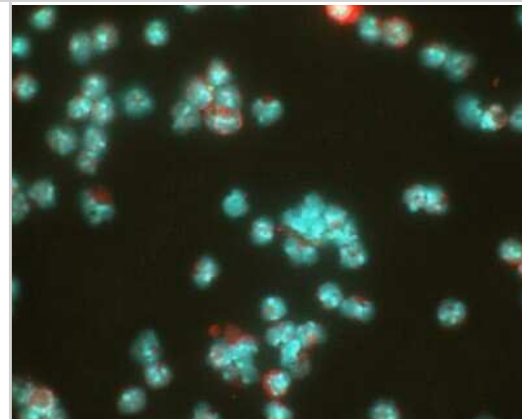
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of HIF-1 alpha in paraffin-embedded mouse kidney tissue section using anti-HIF-1 alpha antibody. Image from verified customer review.



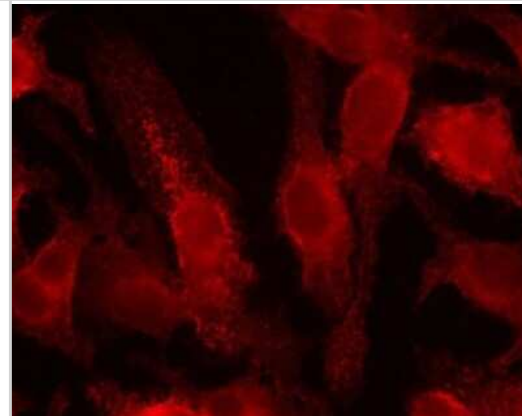
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Nuclear HIF-1 alpha protein expression. Xenografts were established in female nude mice (BALB c[*nu/nu*]) by subcutaneous injection of head and neck squamous cell carcinoma cell lines UT-SCC-2 (UT-2) and UT-SCC-14 (UT-14). Cetuximab (1 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.



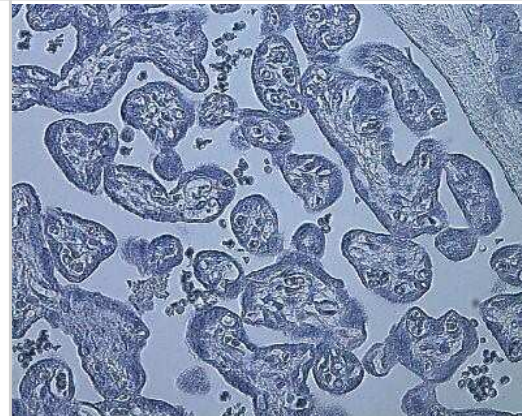
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with *S. aureus*, using NB100-131. Blue: DAPI nuclear staining. Image courtesy of Susan Alexander and Hattie Gresham,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



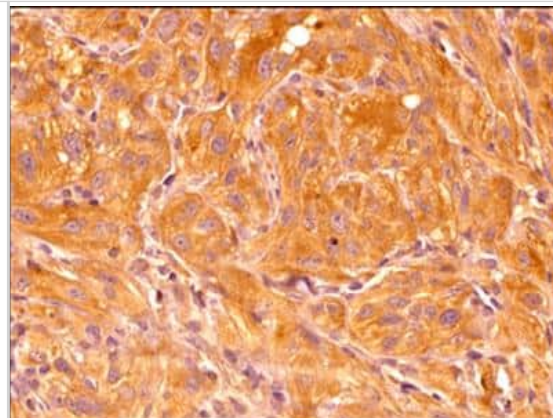
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line. 100X magnification. Image courtesy of Susan Alexander and Hattie Gresham, PhD.



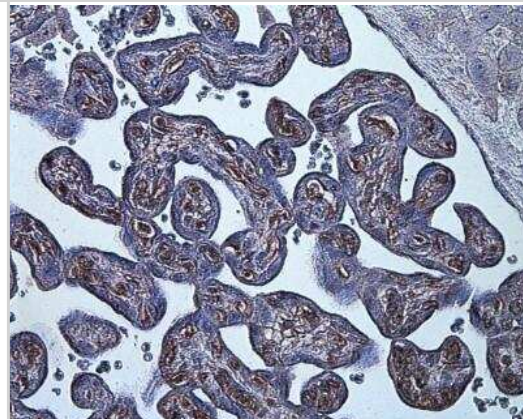
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Negative control stain of human placenta (from sea level) using mouse IgG at 1:100. 4uM paraffin-embedded section.



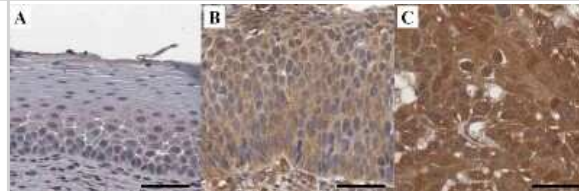
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Analysis of a FFPE tissue section of human renal cancer xenograft using HIF-1 alpha antibody (NB100-131 Lot 83115) at 1:200 dilution. The antibody generated a strong cytoplasmic staining mainly in the cancer cells. Only a fraction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



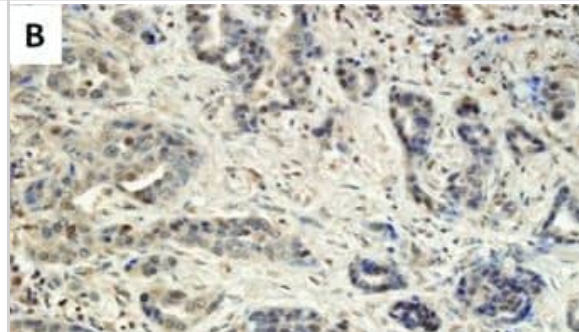
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - HIF-1 alpha staining in hypoxia-induced human placenta.



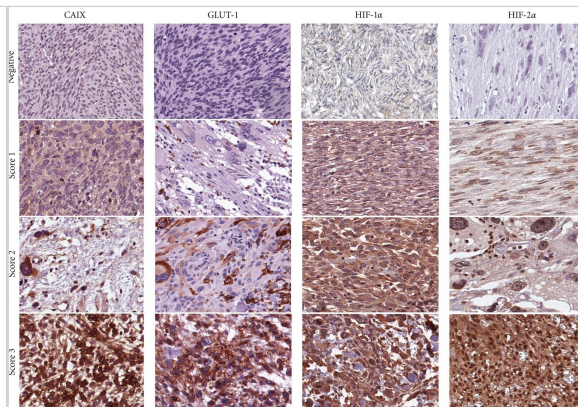
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Representative immunohistochemical expression for HIF-1alpha, c-Met, CA9 and GLUT1. HIF-1alpha is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), and strong staining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



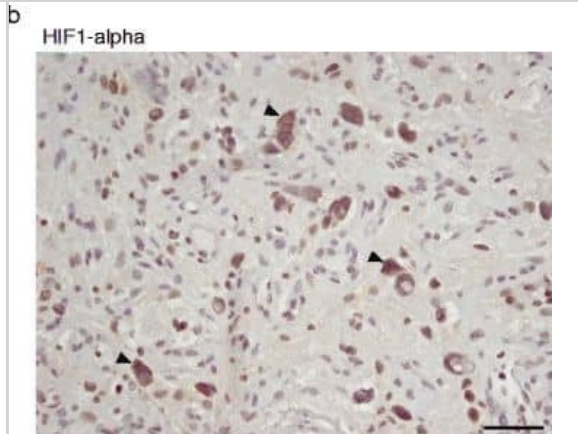
Positive immunohistochemical staining for (A) VEGF, (B) HIF-1 α , (C) DII4 (tumor cells), (D) DII4 (endothelial cells), and (E) CD31 (for microvessel counting, $\times 200$ magnification).



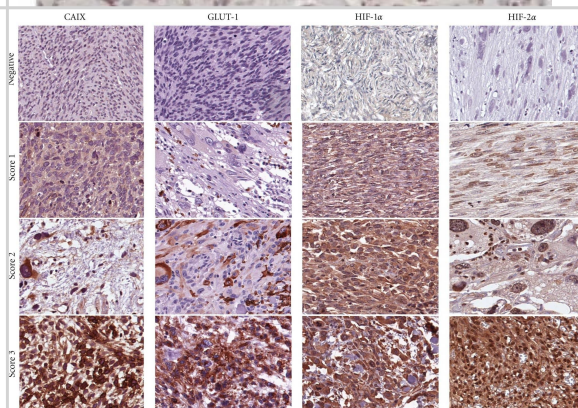
Immunohistochemical analysis in non-GIST STS representing negative, and score 1–3 of CAIX, GLUT-1, HIF-1 α , and HIF-2 α . non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, CAIX: carbonic anhydrase IX, GLUT-1: glucose transporter-1, and HIF-1/2 α : hypoxia induced factor 1/2 α .



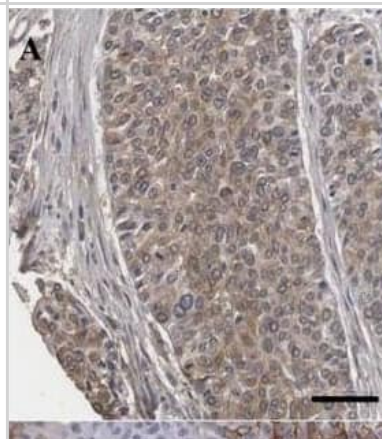
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Histologically distinct cell types in hemangioblastomas do not arise from a common ancestral clone. “Representative images of sample SH-0622 acquired at 400x of (a) H + E & IHC for (b) HIF1- α , (c) VEGF, & (d) PDGFR- β reveal heterogenous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



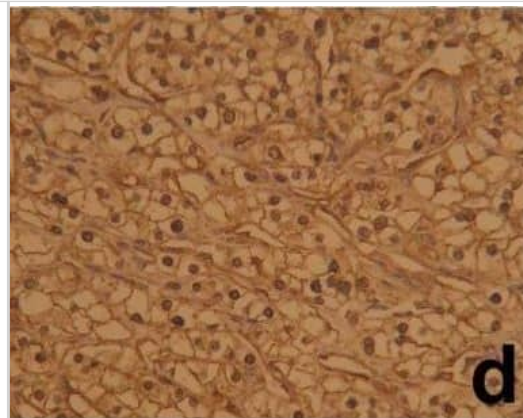
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Immunohistochemical analysis in non-GIST STS representing negative, & score 1–3 of CAIX, GLUT-1, HIF-1 α , & HIF-2 α . non-GIST STS: non-gastrointestinal stromal tumor soft-tissue sarcomas, CAIX: carbonic anhydrase IX, GLUT-1: glucose transporter-1, & HIF-1/2 α : hypoxia induced factor 1/2 α . 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.



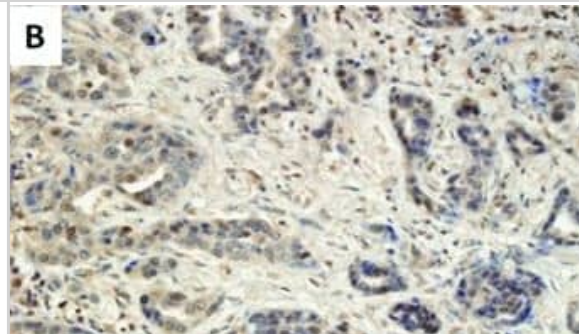
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Digital image analysis of cytoplasmic & membranous staining. Cytoplasmic HIF-1 α staining is shown (A) & automated image analysis utilizing TissuelA recognizes cytoplasmic HIF-1 α staining highlighted in green color (B). CA9 is shown 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 μ 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.



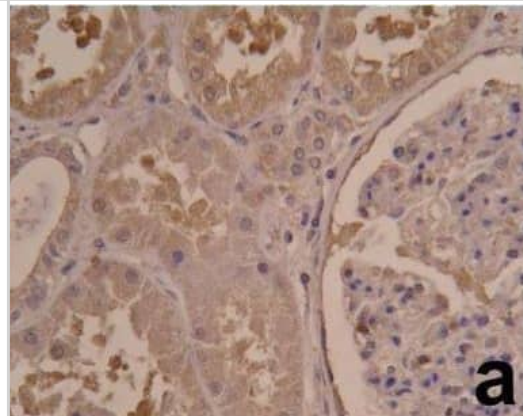
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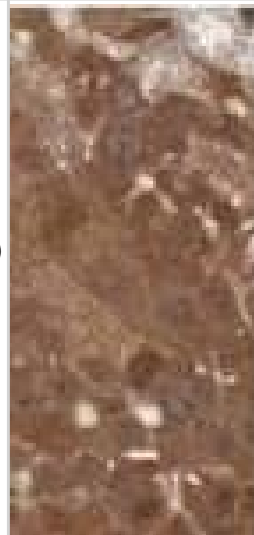
Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Positive immunohistochemical staining for (A) VEGF, (B) HIF-1 α , (C) DII4 (tumor cells), (D) DII4 (endothelial cells), & (E) CD31 (for microvessel counting, $\times 200$ magnification). Image collected & cropped by CiteAb from the following publication (<https://www.spandidos-publications.com/10.3892/or.2012.2075>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



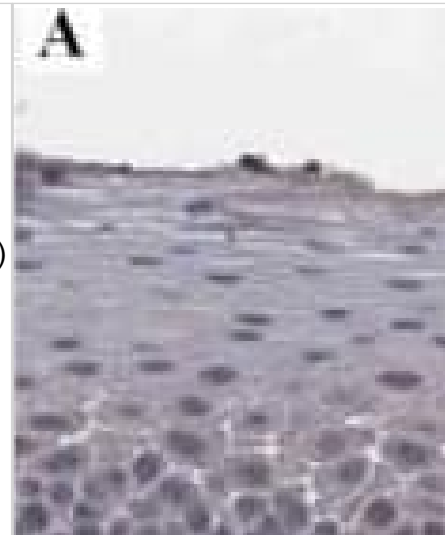
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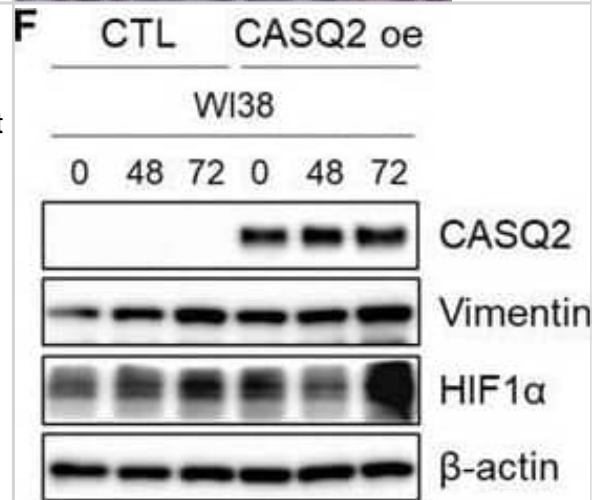
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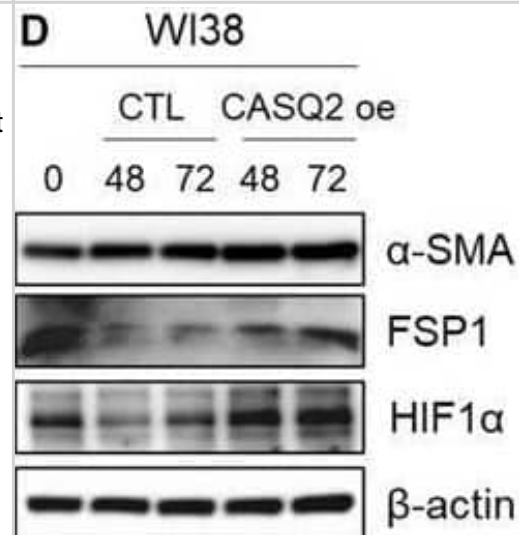
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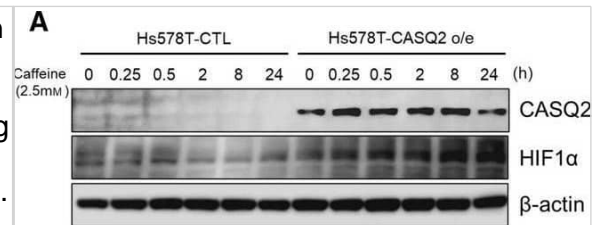
CASQ2 affects the TME and invasiveness through HIF1 α . (A) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the mouse genome (Differentially expressed genes, DEGs; n = 530). (B) The heatmap for average of expression (FPKM) value of most significant differentially expressed genes of angiogenesis and the HIF1 α signaling pathway. (C) The mRNA level of Hif1a, Pgf, Flt1, Kdr, Flt4, and Nos3 in Hs578T CTL and CASQ2 o/e tumors (means \pm SEM, n = 3; ***P < 0.001 by the multiple t-test). (D, E) Expression level of α -SMA, FSP, and HIF1 α in WI38 cells after indirect coculture with Hs578T CTL or Hs578T CASQ2 o/e cells (means \pm SEM, n = 3; *P < 0.05 by the multiple t-test). (F, G) Expression level of CASQ2 and vimentin in Hs578T CTL or Hs578T CASQ2 o/e cells indirectly cocultured with WI38 (means \pm SEM, n = 3; *P < 0.05 by the multiple t-test). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/34743414>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



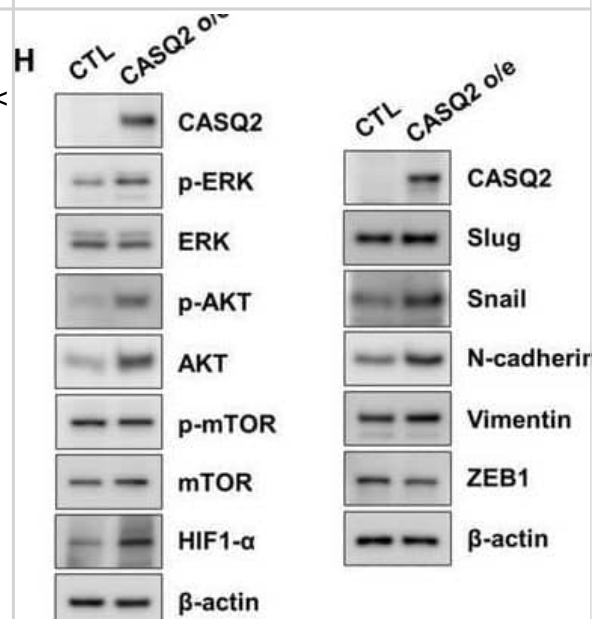
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CASQ2 affects the tumor invasiveness through HIF1 α . (A, B) Expression level of HIF1 α in caffeine-treated Hs578T cells with or without overexpression of CASQ2 (means \pm SEM, n = 3; *P < 0.05, **P < 0.01 by the Mann–Whitney U-test). (C) Confocal microscopy images showing F-actin in Hs578T cells overexpressing CASQ2 under hypoxic conditions (**P < 0.01 by the Mann–Whitney U-test). Scale bar = 10 μ m. (D) Proliferation rate of control (CTL) and CASQ2-overexpressing (CASQ2 o/e) breast cancer cells under normoxic and hypoxic conditions (mean \pm SEM, n = 3; *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by ANOVA with Tukey's post hoc test). (E, F) Hematoxylin and eosin staining of lung tissue sections from Hs578T-CTL or CASQ2-derived tumors (mean \pm SEM, n = 5; *P < 0.05 and **P < 0.01 by two-tailed Student's t-test). Scale bar = 100 μ m. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/34743414>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



CASQ2 induces phenotypic changes in breast cancer cells. (A) Relationship between the proliferation rate and overexpression of CASQ2 in breast cancer cell lines (mean \pm SEM, n = 3; *P < 0.05, **P < 0.01, and ***P < 0.001 using the multiple t-test). (B) Migration and invasion rates of Hs578T cells (mean \pm SEM, n = 3; **P < 0.01 using two-tailed Student's t-test). Scale bar = 50 μ m. (C) Three-dimensional culture of Hs578T cells (mean \pm SEM, n = 3; **P < 0.01 using two-tailed Student's t-test). Scale bar = 100 μ m. (D) Tumorsphere culture of Hs578T cells (mean \pm SEM, n = 3; **P < 0.01 using two-tailed Student's t-test). Scale bar = 100 μ m. (E) Expression of CD44, CD24, and ALDH1 cancer stem cell markers in adherent and tumorsphere cultures of Hs578T cells. (F) Measurement of intracellular Ca²⁺ in Hs578T cells using a calcium crimson reagent. Cells were loaded with the calcium indicator calcium crimson (5 μ m) for 30 min, and then, BAPTA-AM (10 μ m) was added. After washing, the cells were stimulated by treatment with 5 μ m caffeine to measure the intracellular calcium concentration at 360 s (mean \pm SEM, n = 3; *P < 0.05 and **P < 0.01 after ANOVA with Tukey's post hoc test). (G) Effect of lacidipine on the expression of cancer stem cell markers in tumorspheres of breast cancer cells. The figure shows one of three independent experiments. Statistical test result is shown in Fig. S6. (H) Epithelial–mesenchymal transition (EMT)-related protein expression in breast cancer cells. All results are representatives of three independent experiments. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/34743414>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

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

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]

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]

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]

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)

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 dips Xylene #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 in 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 dH₂O.

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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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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