Product Datasheet

ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free
NB100-124

Unit Size: 0.1 ml

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

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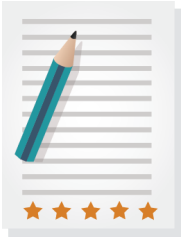
Updated 10/23/2024 v.20.1



NB100-124

ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	H1beta234
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	86.6 kDa

Product Description

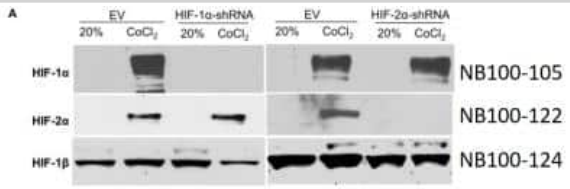
Host	Mouse
Gene ID	405
Gene Symbol	ARNT
Species	Human, Mouse, Rat, Bovine, Ferret, Primate, Sheep
Immunogen	ARNT/HIF-1 beta Antibody (H1beta234) was developed against a fusion protein containing amino acids 496-789 of human HIF-1 beta. [Uniprot: P27540]

Product Application Details

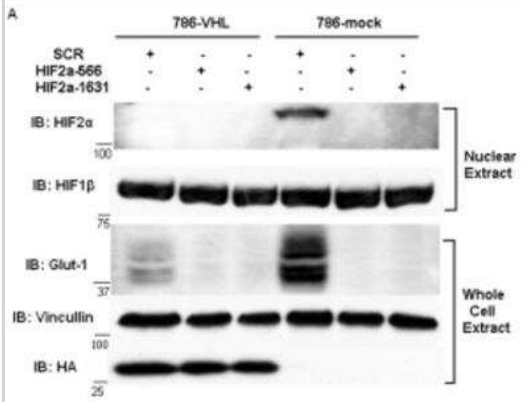
Applications	Western Blot, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Chromatin Immunoprecipitation Sequencing
Recommended Dilutions	Western Blot 1:500, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 25343232), Immunoprecipitation, Immunohistochemistry-Paraffin 1:100, Immunoblotting 1:100 - 1:2000, Gel Super Shift Assays reported in scientific literature (PMID 11325839), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Chromatin Immunoprecipitation Sequencing
Application Notes	In Western blot, a band at approximately 92 kDa is seen.

Images

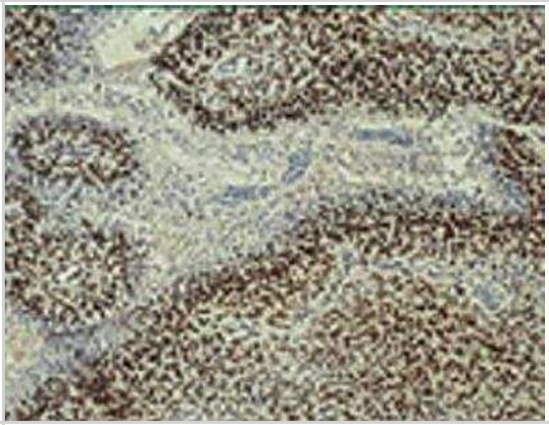
Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - Western Blot analysis with ARNT/HIF-1 beta antibody (H1beta234) [NB100-124], theoretical molecular weight 86.6 kDa. Expression of CD44 mRNA in genetically engineered MDA-MB-231 cells. (A) Immunoblot analysis of HIF-1A or HIF-2A expression in whole cell extracts from MDA-MB-231 cells stably expressing EV, HIF-1A-shRNA or HIF-2A-shRNA under normoxia or in response to 4 h treatment with 200 uM CoCl2. HIF-1B expression was used as a loading control. Image collected and cropped by CiteAb from the following publication (//dx.plos.org/10.1371/journal.pone.0044078) licensed under a CC-BY license.



Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - HIF was not the only factor stabilizing activated EGFR in VHL-deficient ccRCC cells. A. Western blot analysis of 786-VHL and 786-mock cells stably expressing shRNA constructs. For HIF2A (NB100-480) and ARNT/HIF-1 beta antibody (H1beta234)[NB100-124] analysis, nuclear extracts were generated and analyzed. Anti-HA blot detected HA-VHL. Means and SDs of three separate experiments were shown. Theoretical molecular weight for ARNT/HIF-1 beta is 86.6 kDa, observed molecular weight ~90 kDa. Image collected and cropped by CiteAb from the following publication (//dx.plos.org/10.1371/journal.pone.0023936) licensed under a CC-BY license.

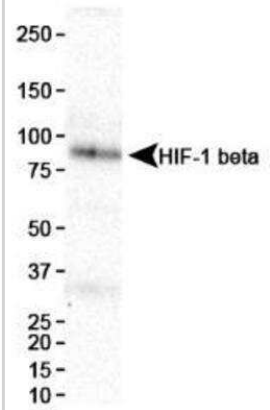


Immunohistochemistry: ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - Staining of human glioblastoma multi-forme utilizing ARNT/HIF-1 beta antibody (H1beta234) [NB100-124].

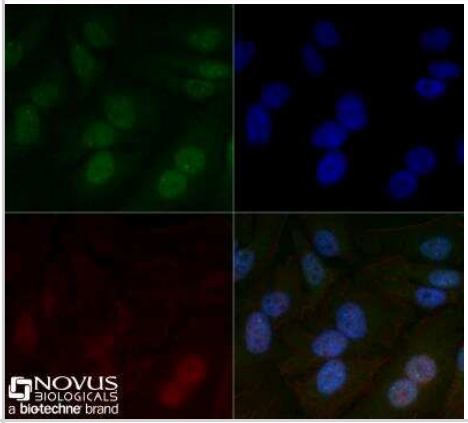


Immunocytochemistry/Immunofluorescence: ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - Co-localization of HIF-2alpha with ARNT/HIF-1 beta antibody (H1beta234) [NB100-124]. ICC/IF detecting indicated proteins using antibodies labelled with Alexa Fluor 555 (red, pseudocolour) and Alexa Fluor 488 (green, pseudocolour). 'Merge' is the red image superimposed onto the green image of the co-stained nuclear proteins. 'Coloc' is the co-localization channel calculated using ImageJ plugin. White indicates pixels where both red and green signal is found (i.e. co-localization). 'Overlay' is the co-localization image superimposed onto the merged image. Inlay is the magnified region (white square). White arrows highlight regions of co-localization. Scale bar, 5 um. Abbreviations: RNAPII, RNA Polymerase II phospho-serine 5. Image collected and cropped by CiteAb from the following publication (https://rsob.royalsocietypublishing.org/lookup/doi/10.1098/rsob.160195), licensed under a CC-BY license.

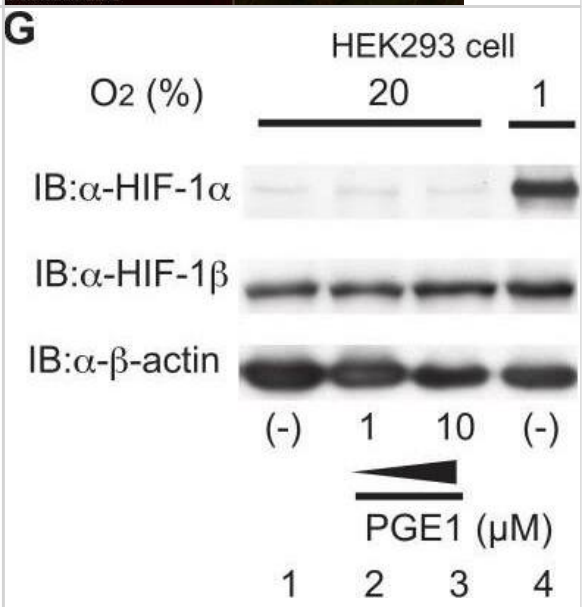
Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - Analysis of HIF-1 beta in HeLa nuclear extract using ARNT/HIF-1 beta antibody (H1beta234) [NB100-124]. Theoretical molecular weight 86.6 kDa. Observed molecular weight ~85 kDa.



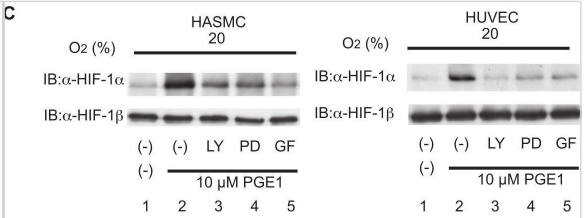
Immunocytochemistry/Immunofluorescence: ARNT/HIF-1 beta Antibody (H1beta234) [NB100-124] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with ARNT/HIF-1 beta antibody (H1beta234) [NB100-124] at 5 ug/mL overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - PGE1 induces HIF-1α protein accumulation in vascular-derived cells.Human aortic smooth muscle cells (HASMCs) (A & B), human umbilical vein endothelial cells (HUVECs) (C & D), & HEK293 cells (G) were exposed to 1 or 10 μM PGE1 under 20% O2 or 1% O2 conditions for 4 h. After treatment, cells were harvested & whole-cell lysates were subjected to immunoblot assay for HIF-1α, HIF-1β, & β-actin protein expression. Experiments were repeated thrice (A, C & G). Representative immunoblots are shown (A & C). Band intensities were analyzed densitometrically (B & D). Fold induction relative to lane 1 was plotted as mean ± S.D. □P < 0.05 compared with the control. HASMCs (E) & HUVECs (F) were exposed to 1 μM PGE1 for the indicated times under 20% O2 & were harvested for immunoblot assay for HIF-1α protein. Experiments were repeated twice. Representative immunoblots are shown. (H) HASMCs & HUVECs were exposed to 10 μM PGE1 for 4 h under 20% O2 & were harvested for immunoblot assay for HIF-2α protein. Experiments were repeated twice. Representative immunoblots are shown. I. HASMCs were exposed to 1 μM PGE1, lipo-PGE1 & PGE1 -alfadex under 20% O2 conditions for 4 h. After treatment, cells were harvested & whole-cell lysates were subjected to immunoblot assay for HIF-1α. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24349900), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - Effect of PGE1 on stability & synthesis of HIF-1α.(A) Human aortic smooth muscle cells (HASMCs) & human umbilical vein endothelial cells (HUVECs) were exposed to 1 μM PGE1 or 100 μM DFX or incubated for 4 h, & CHX was added to a final concentration of 100 μM. The cells were incubated for 0 to 30 min, & whole-cell lysates were subjected to immunoblot assay using anti-HIF-1α or -β antibodies. (B) Serum-starved HASMCs were pretreated with no drug & 1 μM PGE1 for 30 min in Met-free medium. [35S]Met-Cys was added, & the cells were incubated for 60 min prior to preparation of cell lysates. Aliquots of 1 mg of the lysates were subjected to immunoprecipitation with anti-HIF-1α antibody, separated by SDS-PAGE & exposed. Aliquots of 50 μg of the same lysate were separately subjected to immunoblotting analysis with anti-β-actin antibody. (C) HASMCs & HUVECs were exposed to vehicle or 1 μM PGE1 for 4 h in the presence of 10 μM LY294002 (LY), 50 μM PD98059 (PD), or 5 μM GF109203X (GF). The cells were harvested & the whole-cell lysates were subjected to immunoblot assay for HIF-1α & β-actin protein expression. Experiments were repeated at least twice. Representative immunoblots are shown. (D) HASMCs were exposed to vehicle or 1 μM PGE1 for 12 h in the presence of 10 μM LY294002 (LY), 50 μM PD98059 (PD), or 5 μM GF109203X (GF). Cells were harvested & subjected to semi-quantitative RT-PCR for VEGF & GLUT1. Experiments were repeated three times. Fold induction relative to that under 20% O2 without PGE1 treatment was plotted. □P < 0.05 compared with the control (20%, PGE1 treatment without any kinase inhibitors). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24349900), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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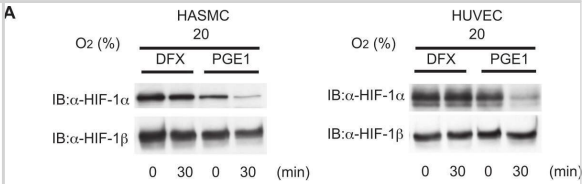

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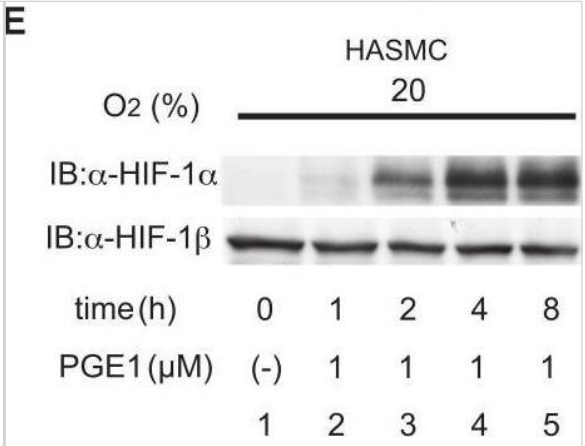
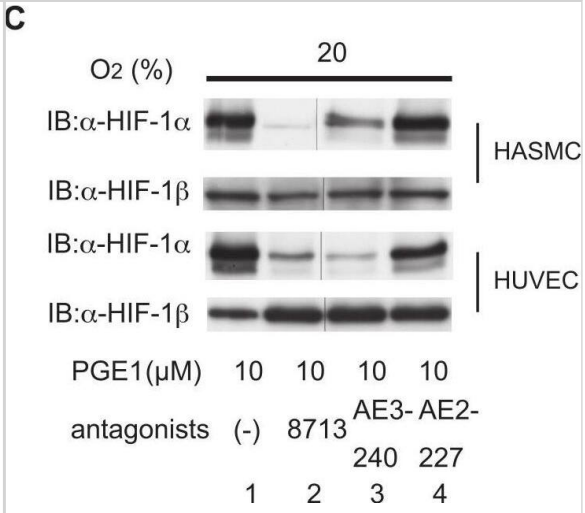


Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - Differential involvement of EP receptors in PGE1-induced HIF-1α protein accumulation.(A) Expression of EP1, EP2, EP3, & EP4 receptors in human aortic smooth muscle cells (HASMCs), human umbilical vein endothelial cells (HUVECs), & cells of the neuroblastoma cell line SH-SY5Y. HASMCs, HUVECs, & SH-SY5Y cells were cultured under 20% O2 & harvested for semi-quantitative RT-PCR for EP1-4 receptors. Experiments were repeated at least three times in triplicate. Fold induction relative to expression in SH-SY5Y cells was plotted as mean ± S.D. HASMCs & HUVECs were exposed to 1 μM of EP-receptor-specific agonists (ONO-DI-004 for EP1, ONO-AE1-259-01 for EP2, ONO-AE-248 for EP3, & ONO-AE1-329 for EP4) for 4 h (B). HASMCs & HUVECs were exposed to 1 μM PGE1 with or without 1 μM EP-receptor-specific antagonists (ONO-8713 against EP1, ONO-AE3-240 against EP3, & ONO-AE2-227 against EP4) for 4 h (C). The cells were harvested & the whole-cell lysates were subjected to immunoblot assay for HIF-1α & β-actin protein expression. Experiments were repeated twice. Representative immunoblots are shown. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24349900), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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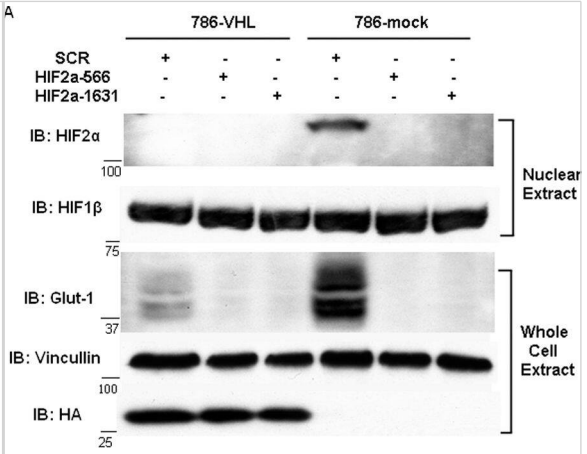
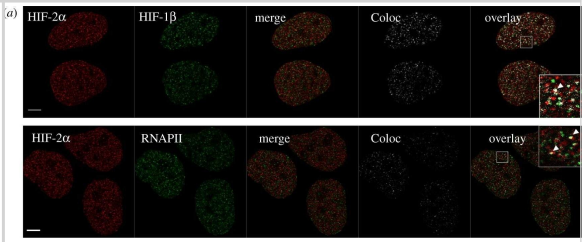


Immunocytochemistry/ Immunofluorescence: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - Co-localization of HIF-2α with HIF-1β & RNAPII. (a) Immunofluorescence detecting indicated proteins using antibodies labelled with Alexa Fluor 555 (red, pseudocolour) & Alexa Fluor 488 (green, pseudocolour). 'Merge' is the red image superimposed onto the green image of the co-stained nuclear proteins. 'Coloc' is the co-localization channel calculated using ImageJ plugin Co-localization Threshold. White indicates pixels where both red & green signal is found (i.e. co-localization). 'Overlay' is the co-localization image superimposed onto the merged image. Inlay is the magnified region (white square). White arrows highlight regions of co-localization. Scale bar, 5 µm. Abbreviations: RNAPII, RNA Polymerase II phospho-serine 5. (b) Immunofluorescence images were analysed using ImageJ plugin Co-localization Threshold with use of the Costes et al. [31] method to automatically create a threshold prior to calculating the Mander's coefficient for both proteins. The results are given as, for example, the percentage of protein A (HIF-2α) that co-localized with protein B (HIF-1β or RNAPII) & vice versa. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27655733), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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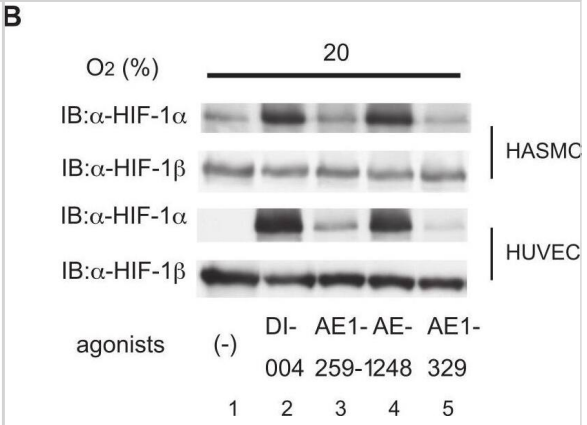
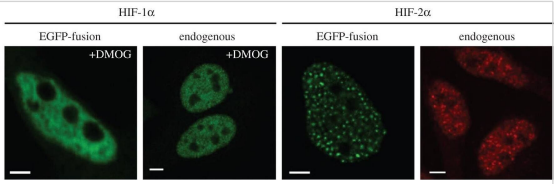
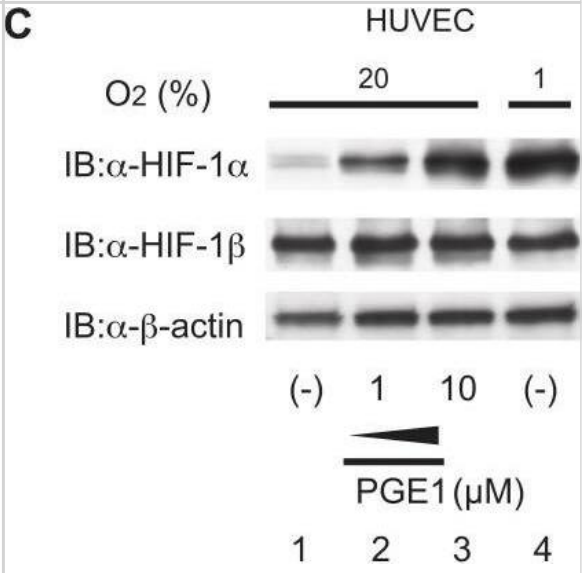


Immunocytochemistry/ Immunofluorescence: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - Sub-nuclear localization of HIF-1α & HIF-2α. (a) HeLa cells ectopically expressing HIF-1α & HIF-2α EGFP fusions compared with endogenous HIF-1α & HIF-2α labelled using immunostaining. Images of HIF-1α were taken following DMOG treatment (6 h; 0.5 mM). Scale bar, 5 μm. (b) HeLa cells transiently transfected with plasmids encoding (i) clover-HIF-2α (green, pseudocolour), (ii) dsRED-HIF-2α (red, pseudocolour), (iii) HIF-2α-venus (yellow pseudocolour) & (iv) Halotag-HIF-2α (green, pseudocolour). The cells expressing Halotag-HIF-2α were labelled with the fluorescent Oregon Green Halotag ligand (HL-OregonGreen; Promega, WI, USA) to visualize the fusion protein. (c) Confocal images of C2C12 (mouse myoblast; top) & HEK293T (Human embryonic kidney cells; bottom) cells ectopically expressing EGFP-HIF-2α. Scale bar, 5 μm. (d) HeLa cells transiently transfected with EGFP-HIF-2α were imaged with a CCD camera. One thousand frames were acquired per cell in normoxia, hypoxia (1% v/v O2, 16 h) or following treatment with DMOG (0.5 mM, 6 h). The average (±s.d.) number of speckles per nucleus in each condition was 64 ± 49 (n = 25), 44 ± 24 (n = 24) & 96 ± 33 (n = 22), respectively. Mean of the sample data represented by the red dashed line. (e) Using the images from (d) the average speckle area per nucleus over the 1000 frames. The mean values (±s.d.) for each condition were 0.24 ± 0.09 μm (n = 25), 0.21 ± 0.07 μm (n = 24) & 0.27 ± 0.09 μm (n = 22), respectively. The mean values for hypoxia & DMOG were compared with the normoxic values (independent t-test, significance value set at 5%). Mean of the sample data represented by the red dashed line. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27655733), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: ARNT/HIF-1 beta Antibody (H1beta234) - BSA Free [NB100-124] - PGE1 induces HIF-1α protein accumulation in vascular-derived cells.Human aortic smooth muscle cells (HASMCs) (A & B), human umbilical vein endothelial cells (HUVECs) (C & D), & HEK293 cells (G) were exposed to 1 or 10 μM PGE1 under 20% O2 or 1% O2 conditions for 4 h. After treatment, cells were harvested & whole-cell lysates were subjected to immunoblot assay for HIF-1α, HIF-1β, & β-actin protein expression. Experiments were repeated thrice (A, C & G). Representative immunoblots are shown (A & C). Band intensities were analyzed densitometrically (B & D). Fold induction relative to lane 1 was plotted as mean ± S.D. □P < 0.05 compared with the control. HASMCs (E) & HUVECs (F) were exposed to 1 μM PGE1 for the indicated times under 20% O2 & were harvested for immunoblot assay for HIF-1α protein. Experiments were repeated twice. Representative immunoblots are shown. (H) HASMCs & HUVECs were exposed to 10 μM PGE1 for 4 h under 20% O2 & were harvested for immunoblot assay for HIF-2α protein. Experiments were repeated twice. Representative immunoblots are shown. I. HASMCs were exposed to 1 μM PGE1, lipo-PGE1 & PGE1 -alfadex under 20% O2 conditions for 4 h. After treatment, cells were harvested & whole-cell lysates were subjected to immunoblot assay for HIF-1α. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24349900), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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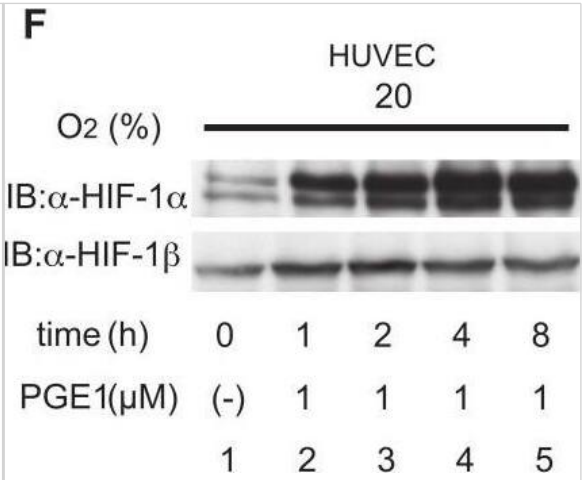

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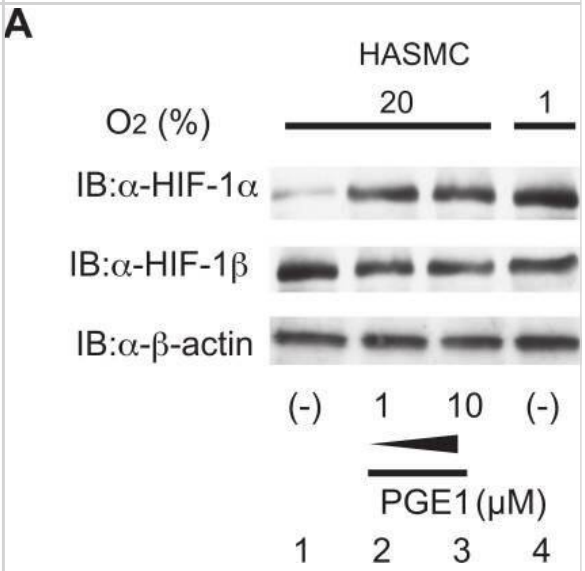

Publications

T Suwa, M Kobayashi, Y Shirai, JM Nam, Y Tabuchi, N Takeda, S Akamatsu, O Ogawa, T Mizowaki, EM Hammond, H Harada SPINK1 as a plasma marker for tumor hypoxia and a therapeutic target for radiosensitization JCI Insight, 2021-11-08;6(21):. 2021-11-08 [PMID: 34747365]

Nan Niu, Hui Li, Xiancai Du, Chan Wang, Junliang Li, Jihui Yang, Cheng Liu, Songhao Yang, Yazhou Zhu, Wei Zhao Effects of NRF-1 and PGC-1α cooperation on HIF-1α and rat cardiomyocyte apoptosis under hypoxia. Gene 2022-06-21 [PMID: 35569770]

Lafleur VN, Halim S, Choudhry H et al. Multi-level interaction between HIF and AHR transcriptional pathways in kidney carcinoma Life science alliance 2023-04-01 [PMID: 36725335]

Haiquan Lu, Yajing Lyu, Linh Tran, Jie Lan, Yangyiran Xie, Yongkang Yang, Naveena L Murugan, Yueyang J Wang, Gregg L Semenza HIF-1 recruits NANOG as a coactivator for TERT gene transcription in hypoxic breast cancer stem cells. Cell reports 2022-02-10 [PMID: 34592152]

Peter W T Lee, Tatsuya Suwa, Minoru Kobayashi, Hui Yang, Lina R Koseki, Satoshi Takeuchi, Christalle C T Chow, Takaaki Yasuhara, Hiroshi Harada Hypoxia- and Postirradiation reoxygenation-induced HMHA1/ARHGAP45 expression contributes to cancer cell invasion in a HIF-dependent manner. British journal of cancer 2024-05-13 [PMID: 38740970]

Chen Y, Cattoglio C, Dailey GM et al. Mechanisms governing target search and binding dynamics of hypoxia-inducible factors eLife 2022-11-02 [PMID: 36322456] (Chip Cytometry, Human)

Tang J, Wu Z, Wang X et al. Hypoxia-Regulated lncRNA USP2-AS1 Drives Head and Neck Squamous Cell Carcinoma Progression Cells 2022-10-28 [PMID: 36359803] (WB, Human)

Simmler P, Cortijo C, Koch LM et al. SF3B1 facilitates HIF1-signaling and promotes malignancy in pancreatic cancer Cell reports 2022-08-23 [PMID: 36001976] (WB, Chemotaxis)

Sasagawa T, Nagamatsu T, Yanagisawa M Et al. Hypoxia-inducible factor-1 beta is essential for upregulation of the hypoxia-induced FLT1 gene in placental trophoblasts Molecular human reproduction 2021-10-19 [PMID: 34665260] (ICC/IF, Human)

Mehibel M, Xu Y, Li CG et al. Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination-deficient cancer models The Journal of clinical investigation 2021-06-01 [PMID: 34060485] (WB, Human)

Tiwari A, Tashiro K, Dixit A Et Al. Loss of HIF1A From Pancreatic Cancer Cells Increases Expression of PPP1R1B and Degradation of p53 to Promote Invasion and Metastasis Gastroenterology 2020-08-03 [PMID: 32768595] (Mouse)

Luo W, Chen I, Chen Y et al. PRDX2 and PRDX4 are negative regulators of hypoxia-inducible factors under conditions of prolonged hypoxia Oncotarget. 2016-02-09 [PMID: 26837221] (Chemotaxis, Human)

Details:
The mechanism for feedback inhibition of hypoxia-inducible factors during prolonged hypoxia is clarified through studying the interaction of PRDX2 and PRDX4 with HIF-1 alpha and HIF-2 alpha.

More publications at http://www.novusbio.com/NB100-124

Procedures

Protocol specific for HIF-1 beta Antibody (NB100-124)

Western Blot Procedure

1. Resolve aliquots (15 mg) of induced nuclear protein extracts on a SDS/6% polyacrylamide gel.
2. Transfer to nitrocellulose membranes in 20 mM Tris-HCl (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membranes for 1.5 hours with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4. Incubate membranes for 1.5 hours at room temperature (RT) in NB 100-124 diluted 1:1,500 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 membranes 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
24.2g 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)
Incubate membrane for 30 minutes at 56 degrees C. Wash membrane 15 minutes with several changes 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-1beta has a very short half-life. Whole cell extracts or nuclear extracts of hypoxia induced cell lines (293, Hep3B, COS7, Hepa) are useful as a positive control. Nuclear Extract

Preparation Reference: Wang and Semenza. "Purification and Characterization of Hypoxia-Inducible Factor 1". Journal of Biological Chemistry. 270(3): 1230-1237, 1995.

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Products Related to NB100-124

NBP3-11826	HIF-1 alpha Knockout CoCl2-treated/untreated HeLa Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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.

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