# Product Datasheet

## HIF-1 alpha Antibody (H1alpha67)
## NB100-123SS

Unit Size: 0.025 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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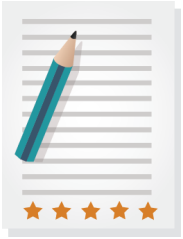
Updated 10/23/2024 v.20.1





# NB100-123SS

HIF-1 alpha Antibody (H1alpha67)

| Product Information | |
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| **Unit Size** | 0.025 ml |
| **Concentration** | 1.0 mg/ml |
| **Storage** | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles. |
| **Clonality** | Monoclonal |
| **Clone** | H1alpha67 |
| **Preservative** | 0.05% Sodium Azide |
| **Isotype** | IgG2b |
| **Purity** | Protein A purified |
| **Buffer** | PBS with 1% BSA |
| **Target Molecular Weight** | 93 kDa |

| Product Description | |
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| **Host** | Mouse |
| **Gene ID** | 3091 |
| **Gene Symbol** | HIF1A |
| **Species** | Human, Mouse, Rat, Porcine, Avian, Bovine, Canine, Ferret, Primate, Rabbit, Sheep |
| **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. Rabbit reactivity reported in scientific literature (PMID: 16738327, 26339038). |
| **Immunogen** | This HIF-1 alpha Antibody (H1alpha67) was developed against a fusion protein containing amino acids 432 - 528 of human HIF-1 alpha [Uniprot# Q16665]. |

| Product Application Details | |
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| **Applications** | Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated |
| **Recommended Dilutions** | Western Blot 1:500 - 1:1000, Chromatin Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature, Flow Cytometry 1:10 - 1:1000, ELISA 1:100 - 1:2000. Use reported in scientific literature, Immunohistochemistry 1:100 - 1:300, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1:10, Immunohistochemistry-Paraffin 1:100 - 1:300, Immunoblotting reported in multiple pieces of scientific literature, Gel Super Shift Assays 1:1 - 1:100. Use reported in scientific literature, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockout Validated reported in scientific literature (PMID 27991597), Knockdown Validated |
| **Application Notes** | By WB, this antibody recognizes bands at 120kDa representing HIF-1 alpha in induced tissues and cells. Multiple bands may be seen at 120kDa representing post-translational modifications. Nuclear extracts are recommended for WB. |

## Images

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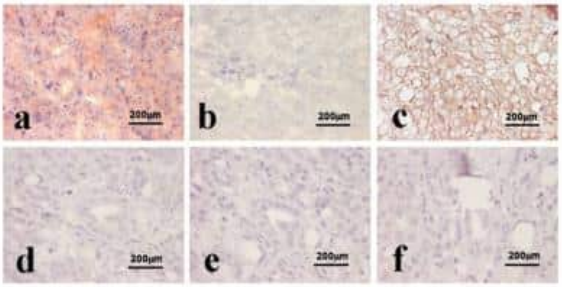
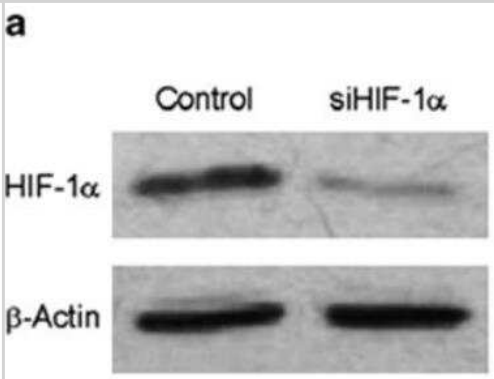
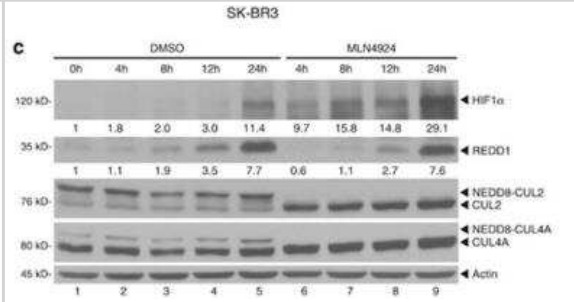
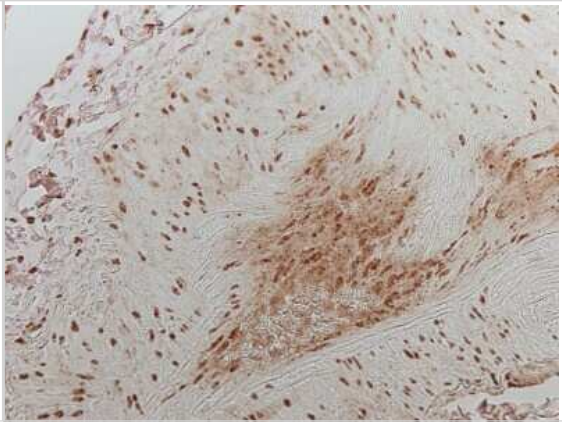
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Results of in situ hybridization and immunohistochemistry on thin adjacent section to detect expression of HIF-1a1.2 mRNA and HIF1a protein in malignant and benign prostate tissue. In situ hybridization (antisense probe, Fig. 3a) and immunostaining with HIF1a Ab2 (Fig. 3c) on thin adjacent sections of NE-differentiated prostate adenocarcinoma showed co-localization of HIF1a1.2 transcript and HIF-1a protein. Incubation with sense probe did not generate any detectable hybridization signals (Fig. 3b). Both In situ hybridization (Fig. 3d antisense) and HIF-1a Ab2 immunostaining (Fig. 3f) were negative in non-NE-differentiated prostate adenocarcinoma. In situ hybridization with sense probe performed on non-NE-differentiated prostate cancer (Fig. 3e). Image collected and cropped by CiteAb from the following publication (https://www.biomedcentral.com/1471-2407/10/385), licensed under a CC-BY license. |  |
| Knockdown Validated: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HIF-1 inhibits the apoptosis of hypoxic glioblastoma cells. U87MG cells were transfected with siRNA against HIF-1alpha. Forty-eight hours after transfection, cells were incubated for 2 h in hypoxia (1% O2). HIF-1alpha was detected by immunoblotting. beta-Actin was used as a loading control. The results are representative for three independent experiments. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2013562) licensed under a CC-BY license. |  |
| Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - MLN4924 induces accumulation of HIF1a in a time dependent manner. Cells were treated with 0.1uM MLN4924 for indicated time periods, followed by IB with indicated antibodies. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2012125) licensed under a CC-BY license. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis of HIF-1 alpha in human lung tissue. Image courtesy of product review by Aneta Gandjeva. |  |

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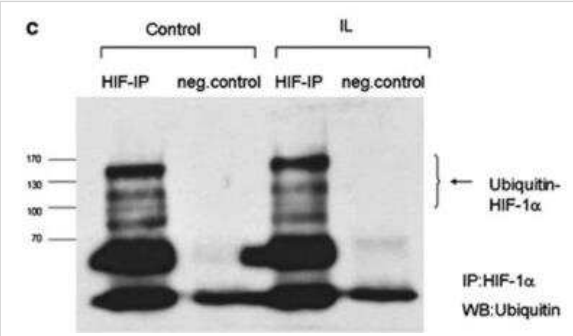
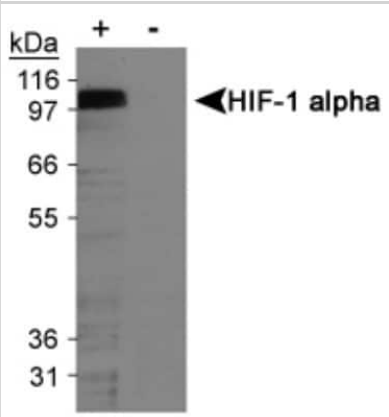
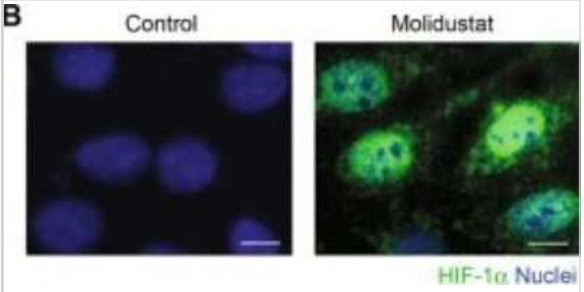
| Immunoprecipitation: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - MG132 attenuates the inhibitory effect of interleukin-1beta on HIF-1/AM axis. U87MG cells were incubated for 2 h in hypoxia (1% O2) with or without interleukin-1beta (10 ng/ml). The proteasome inhibitor MG132 was added 30 min in advance to prevent ubiquitinated HIF-1alpha from proteasomal degradation. HIF-1alpha was precipitated by anti-HIF-1alpha antibody. Ubiquitin was detected by immunoblotting. Non-specific mouse IgG was used as a negative control. The results are representative for three independent experiments Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2013562) licensed under a CC-BY license. |  |
| Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis using the HRP conjugate of NB100-123. Detection of HIF-1 alpha in cobalt chloride treated/untreated COS-7 nuclear extracts using NB100-123. |  |
| Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis using the HRP conjugate of NB100-123. On day 1, MEF cells (+/+,-/-), were grown on 15cm dish (2x10 to the 6th cells). On day 2, cells were exposed to hypoxia for 4hrs. Cells were washed with ice cold PBS twice and whole cell protein was extracted extracted with RIPA buffer fortified with protease. Upon quantification, 100ug of protein was fractionated on 7% polyacralymide gel. Gel was transferred overnight onto nitrocellulose membrane. The membrane was probed with HIF-1 alpha monoclonal antibody at a 1:500 dilution (NB100-123). The secondary antibody was conjugated with HRP and was used at a 1:2500 dilution. Photo courtesy of Dr. Gregg Semenza, Johns Hopkins University. |  |
| Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Dapagliflozin protects against metabolic switch by inhibition of HIF-1 alpha. Representative images of PTCs incubated with 10 umol/l of molidustat for 6 h with immunofluorescent staining for HIF-1 alpha. Scale bar = 10 um. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/33187129/) licensed under a CC-BY license. |  |

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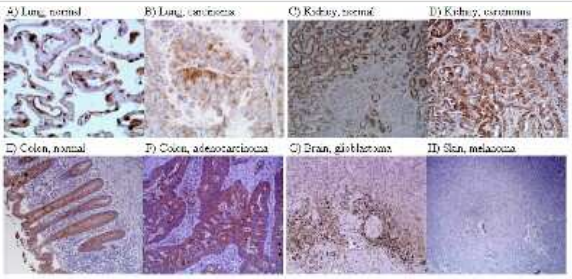
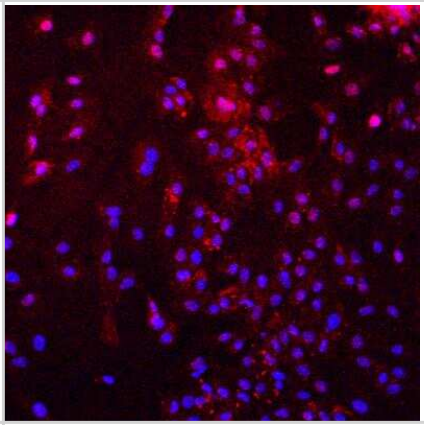
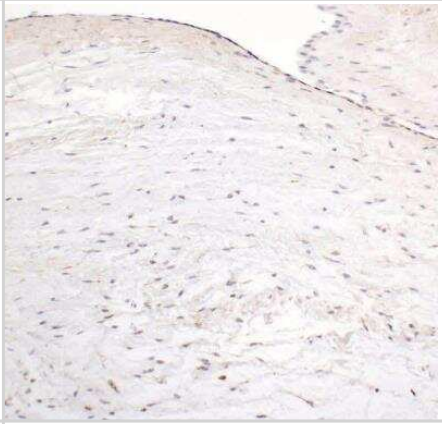
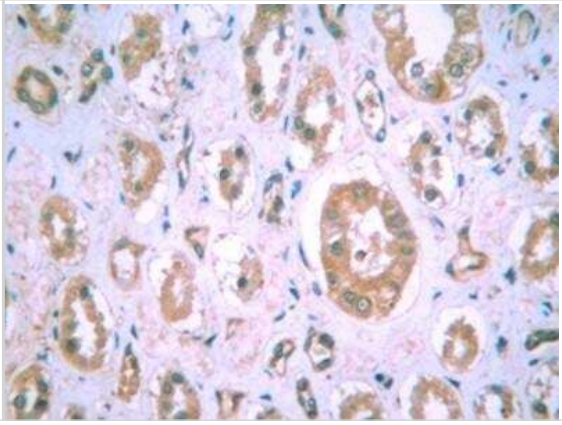
| Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1alpha protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, x400; B, x400; C, x100; D, x100; E, x100; F, x100; G, x40; H, x40. Image collected and cropped by CiteAb from the following publication (https://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. |  |
| Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining in pig endothelial cells under hypoxia condition using NB100-123. Image from verified customer review. |  |
| Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining on pig tissue (brown) using NB100-123. Image from verified customer review. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining of biotin conjugated HIF-1 alpha (NB100-123B) in human kidney. |  |

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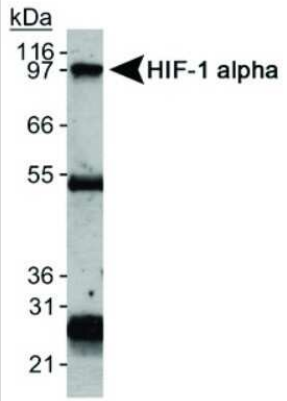
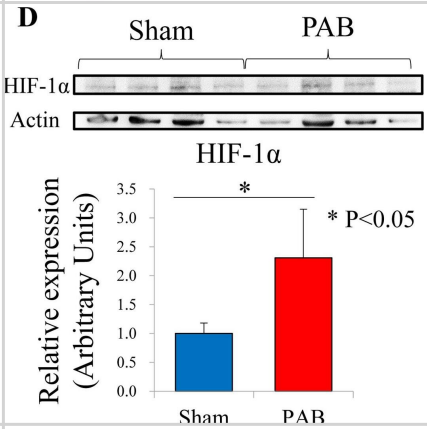
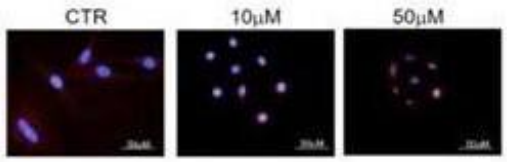
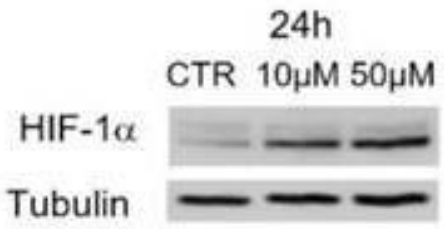
| Immunoprecipitation: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunoprecipitation of HIF-1alpha using NB100-123 in COS7 CoCl2 treated lysate. Heavy and light chains are also detected. Image using the HRP form of this antibody. |  |
| Immunohistological and western blot analyses of HIF-1α.(A) PAB liver stained by HIF-1α (original magnification, ×20). (B) Sham-operated control liver stained by HIF-1α (original magnification, ×20). (C) Enlarged view of the boxed area in the Image (A) (original magnification, ×100). (D) Western blot analysis of HIF-1α (mean ± SEM), * P<0.05. HIF-1α: hypoxia-inducible factor-1α; PAB: pulmonary artery banding. |  |
| Immunocytochemistry/Immunofluorescence: Mouse Monoclonal HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Images of HIF-1 alpha localization (red) in CTR and CoCl2-treated oAECs after 24 h of culture. Image from a verified customer review. |  |
| Western Blot: Mouse Monoclonal HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Image of HIF-1 alpha activation in AECs control cells (CTR) and after 24h of 10 and 50μM of CoCl2-treatment; HIF-1 alpha time course at shorter CoCl2 incubation times. Image from a verified customer review. |  |

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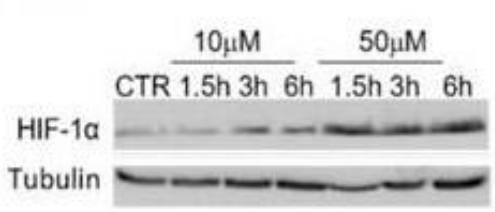
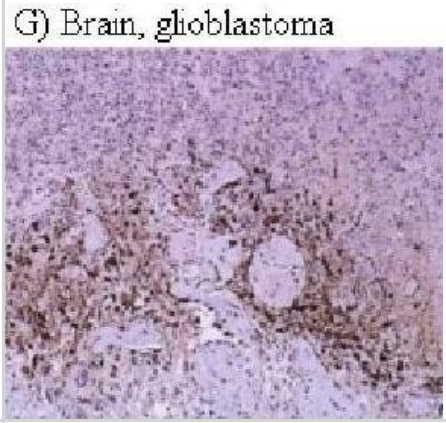
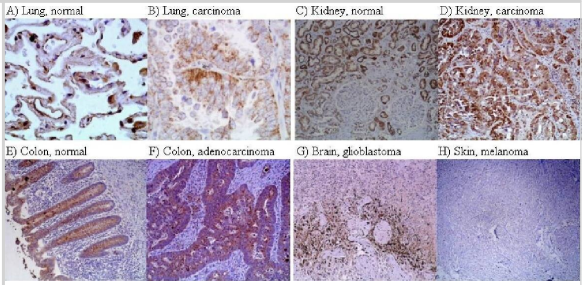
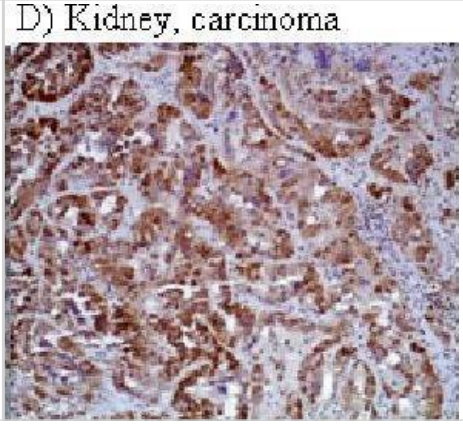
| Western Blot: Mouse Monoclonal HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Image of HIF-1 alpha activation in AECs control cells (CTR) and after 24h of 10 and 50μM of CoCl2-treatment; HIF-1α time course at shorter CoCl2 incubation times. Image from a verified customer review. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |

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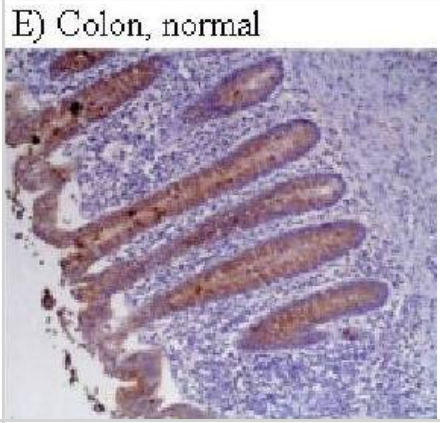
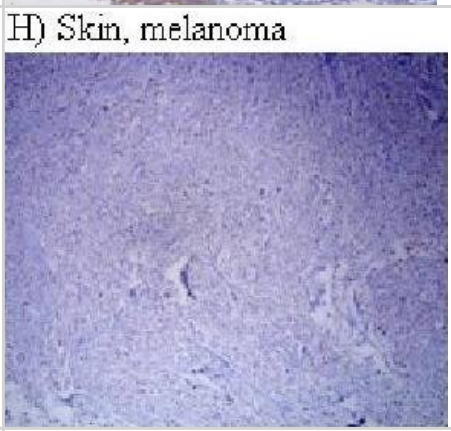
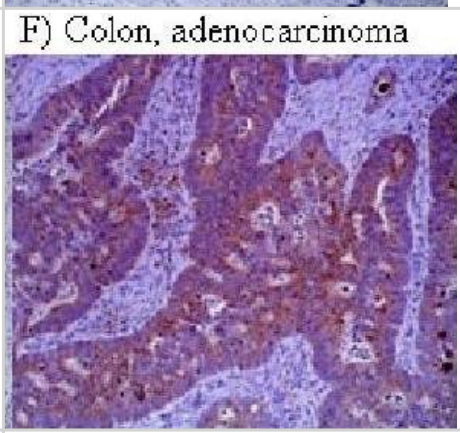
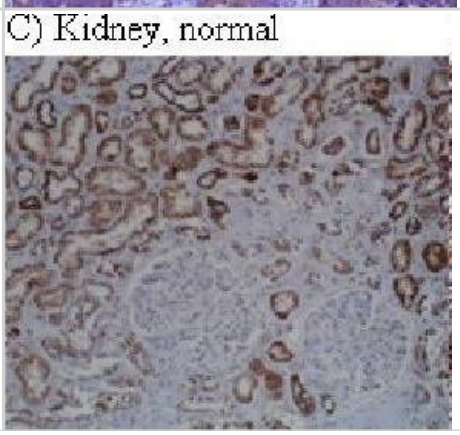
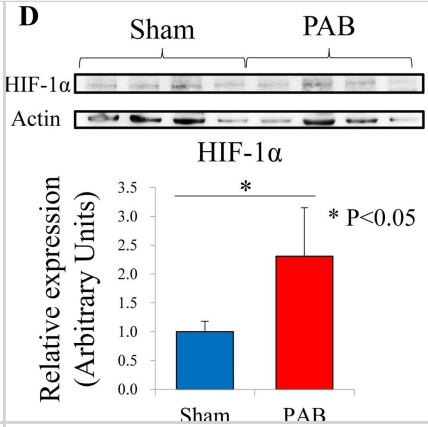
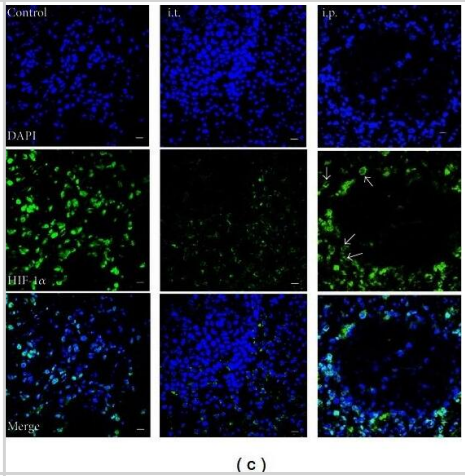
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |

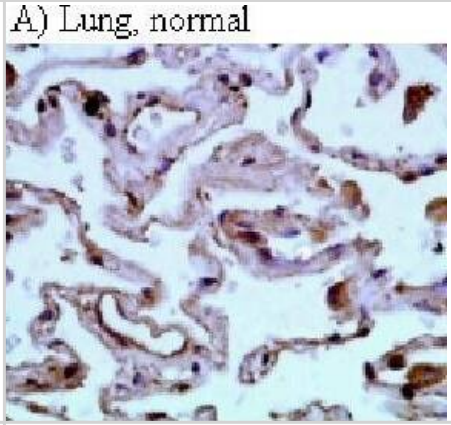
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistological & western blot analyses of HIF-1α.(A) PAB liver stained by HIF-1α (original magnification, ×20). (B) Sham-operated control liver stained by HIF-1α (original magnification, ×20). (C) Enlarged view of the boxed area in the Image (A) (original magnification, ×100). (D) Western blot analysis of HIF-1α (mean ± SEM), * P<0.05. HIF-1α: hypoxia-inducible factor-1α; PAB: pulmonary artery banding. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26863419), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



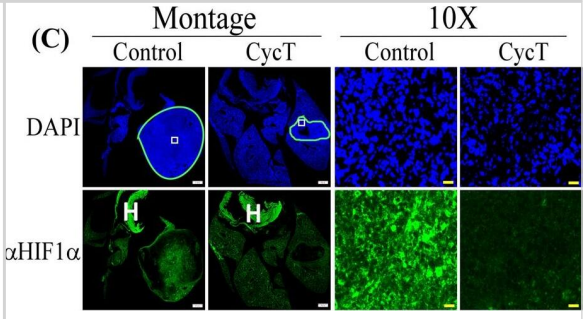

Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis of CBF treatment in HCT116 implanted mouse models & tumour tissue samples. (a) Tumour size versus CBF treatment. The lowest tumour growth rate was found in i.p. group during CBF treatment. (b) Analysis of HIF-1α mRNA level in tumour tissues. There was a significant upregulated HIF-1α level in i.p. group. Results are means with standard errors from 4 replicates. (c) Inhibition of HIF-1α nuclear translocation in the i.t. sample. CBF strongly inhibited HIF-1α nuclear accumulation in the tumour of xenografts implanted with HCT116 cells. Scale bars equal 10 μm. White arrows: HIF-1α in the cytosol. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23818933), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



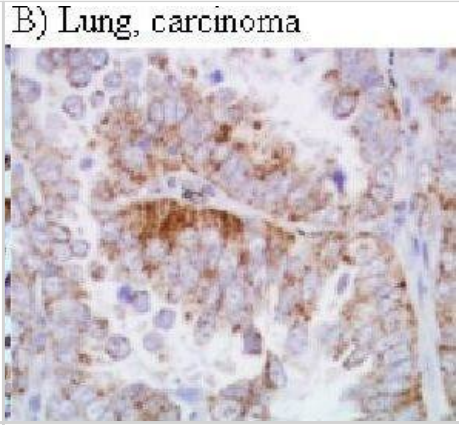

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



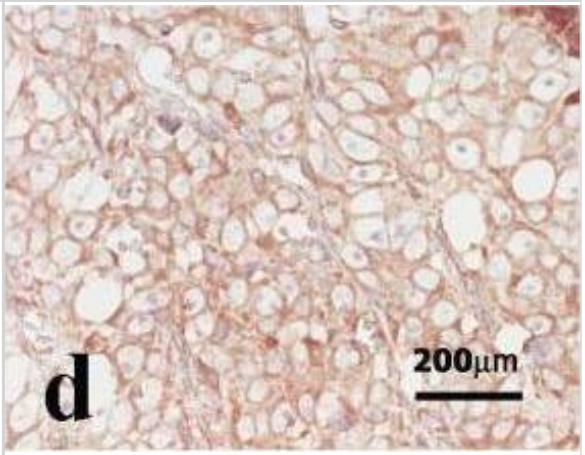

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - CycT alleviates tumor hypoxia. The effects of CycT on the levels of exogenous hypoxia-marker pimonidazole labeling (A), the levels of hypoxia-inducible CA9 enzyme (B), & the levels of hypoxia-inducible factor HIF1α (C) in orthotopic tumor xenografts. Scale bar: Montage, 1 mm; 10X, 20 μm. Data are plotted as mean ± SEM. For statistical analysis, the levels in treated tumors were compared to the levels in control tumors with a Welch 2-sample t-test. **p-value < 0.005. IHC images are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30723259), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



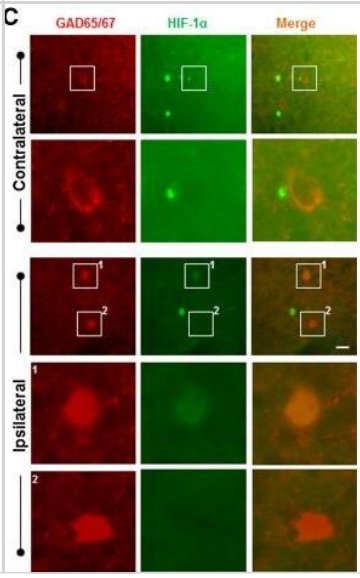
Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected & cropped by CiteAb from the following publication (http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Schematic demonstration of the three HIF1α antibodies used in this study & their epitopes (Figs. 2a & b). Ab1 is a polyclonal antibody raised against N-domain of wild type HIF1α & does not recognize HIF1α1.2 due to it's different N-terminal part (Fig. 2a). Ab2 & Ab3 are monoclonal & polyclonal antibodies, respectively, with epitopes in the common parts of HIF1α & HIF1α1.2 (Fig.2a & b). Immunohistochemical analysis performed on thin adjacent sections of NE-differentiated prostate cancer using the HIF1α antibodies Ab1 (Fig. 2c), Ab 2 (Fig. 2d), Ab 3 (Fig. 2e) & HIF1β antibody (Fig. 2f). Immunopositivity was detected for HIF1α with Ab2 & Ab3 while HIF1α Ab1 produced no detectable staining. HIF1β was also positive in adjacent section (Fig. 2f). Double staining of chromogranin A & androgen receptor antigens on adjacent sections (Fig. 2g) showed immunopositivity for chromogranin A (Fast red). Androgen receptor antibody (DAB, brown) produced no staining. Immunostaining of benign prostate tissue with HIF1α Ab3 showed immunopositivity in NE-like cells of benign prostate tissue (Fig. 2h). In addition, HIF1β antibody recognized benign NE-like cells in benign prostate hyperplasia (Fig. 2i). Double staining with HIF1α Ab3 & HIF1β (Fig. 2j) shows co-localization of the two proteins in NE-like cells of benign prostate hyperplasia (HIF1α Ab3 red stain, HIF1β brown stain). Panels a, b, c, d, e, f & g: 40× objective. Panels h, i & j: 60× objective. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/20663134), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - GAD65/67-positive neurons expressed HIF-1α under hypoxic conditions. A) HIF-1α expression co-localized with GAD65/67-ir in neurons exposed to hypoxia when compared to normoxia (upper panel) or GAD65/67-negative neurons in hypoxia (bottom panel, open arrow). B) Quantification shows the percentage of HIF-1α-expressing GAD65/67-positive neurons after hypoxia in vitro (mean ± SD; from n = 6 cultures). C)In vivo immunostaining illustrates HIF-1α-positive (bottom panel, solid arrow) & HIF-1α-negative (bottom panel, open arrow) in GAD65/67-ir neurons in the ipsilateral region, whereas the contralateral region shows no HIF-1α staining in GAD65/67-ir neurons (see Figure 1 for region selection). Scale bars, 10 µm (A); 20 µm (B). Image collected & cropped by CiteAb from the following publication (https://actaneurocomms.biomedcentral.com/articles/10.1186/2051-5960-2-51), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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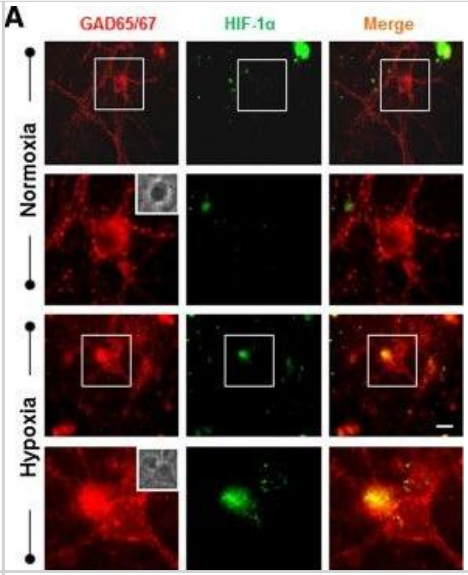
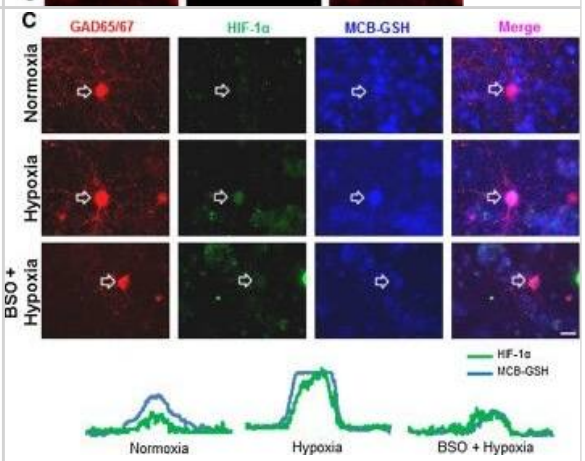
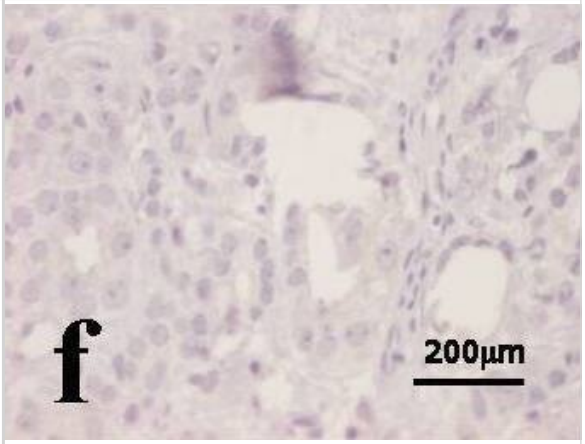
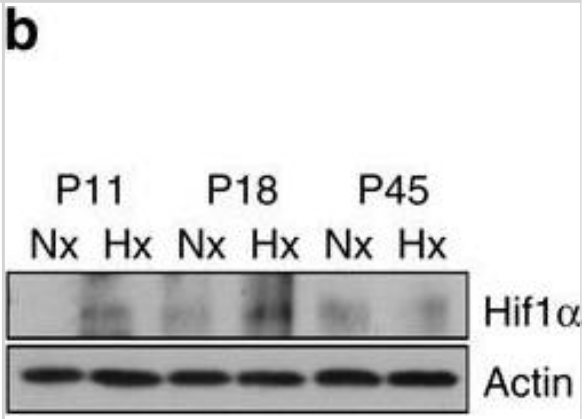
| Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - GAD65/67-positive neurons expressed HIF-1α under hypoxic conditions. A) HIF-1α expression co-localized with GAD65/67-ir in neurons exposed to hypoxia when compared to normoxia (upper panel) or GAD65/67-negative neurons in hypoxia (bottom panel, open arrow). B) Quantification shows the percentage of HIF-1α-expressing GAD65/67-positive neurons after hypoxia in vitro (mean ± SD; from n = 6 cultures). C)In vivo immunostaining illustrates HIF-1α-positive (bottom panel, solid arrow) & HIF-1α-negative (bottom panel, open arrow) in GAD65/67-ir neurons in the ipsilateral region, whereas the contralateral region shows no HIF-1α staining in GAD65/67-ir neurons (see Figure 1 for region selection). Scale bars, 10 µm (A); 20 µm (B). Image collected & cropped by CiteAb from the following publication (https://actaneurocomms.biomedcentral.com/articles/10.1186/2051-5960-2-51), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |
| Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Levels of GSH in cortical neurons exposed to hypoxia. A) During hypoxia, GSH increased in MAP2-ir neurons with round somata (bottom panel, open arrow) & decreased in neurons with pyramidal-like morphology (upper panel, solid arrow). B) GSH increased in a subset of GAD65/67–ir neurons exposed to hypoxia (bottom panel, open arrow). C) Elevated HIF-1α expression in GAD65/67–ir neurons containing high levels of GSH when exposed to hypoxia (middle panel). BSO treatment decreased HIF-1α expression in GAD65/67-positive neurons during hypoxia. D) Percentages of HIF-1α-expressing GAD65/67-ir neurons expressing a high level of GSH & GAD65/67-ir neurons expressing a low levels of GSH & HIF-1α (mean ± SD; n = 3). The “low” level referred to the level of GSH in normoxic GAD65/67 neurons that was normalized to 1. The “high” level referred to the elevated GSH level in GAD65/67 neurons with a mean of 1.40 ± 0.10. E) Total MCB-GSH intensity in GAD65/67-positive neurons with & without BSO after hypoxia (mean ± SD; 8-10 neurons quantified from each experiment, n = 3 independent cultures). Scale bars, 20 µm. Image collected & cropped by CiteAb from the following publication (https://actaneurocomms.biomedcentral.com/articles/10.1186/2051-5960-2-51), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |

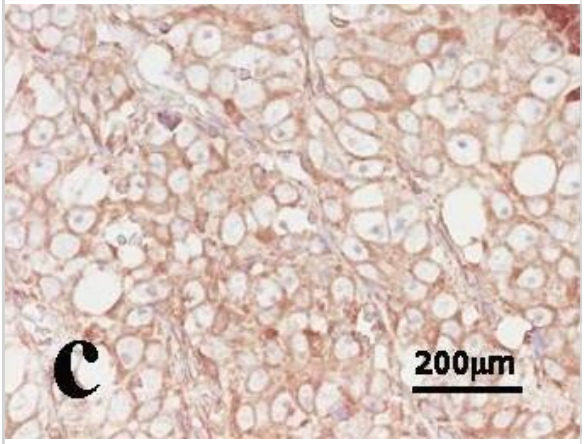
Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Results of in situ hybridization & immunohistochemistry on thin adjacent section to detect expression of HIF-1α1.2 mRNA & HIF1α protein in malignant & benign prostate tissue. In situ hybridization (antisense probe, Fig. 3a) & immunostaining with HIF1α Ab2 (Fig. 3c) on thin adjacent sections of NE-differentiated prostate adenocarcinoma showed co-localization of HIF1α1.2 transcript & HIF-1α protein. Incubation with sense probe did not generate any detectable hybridization signals (Fig. 3b). Both In situ hybridization (Fig. 3d antisense) & HIF-1α Ab2 immunostaining (Fig. 3f) were negative in non-NE-differentiated prostate adenocarcinoma. In situ hybridization with sense probe performed on non-NE-differentiated prostate cancer (Fig. 3e) was negative. In situ hybridizering on benign prostate tissue showed HIF1α1.2 transcript in NE-like cells of benign prostate tissue (Fig. 3g, □). The sense probe on thin adjacent section generated no signals (Fig. 3h,□). Furthermore, co-localization of HIF1α1.2 transcript (Fig. 3i,□,□,□) & HIF1α protein, detected with HIF1αAb3 (Fig. 3j,□,□,□) was also shown in NE-like cells of benign prostate tissue. Panels a, b, c, d, e, f, g, h, i, j: 40× objective. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/20663134), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



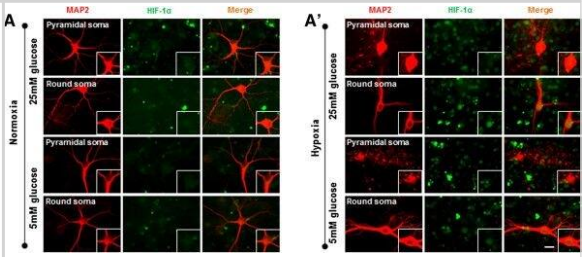

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HX-induced Sirt1 expression in white matter OPCs requires HIF1α.(a) HIF1α stabilization of Sirt1 transcript expression in OPCs as revealed by representative RT–PCR represents higher level of Sirt1 mRNA in VHL cKO mice. GDPDH mRNA serves as a control. Mean±s.e.m., n=3 brains for each group. (b,c) Representative western blot demonstrates a transient increase of HIF1α expression in HX white matter at P18 with no significant effect at P11 (P=0.7955) & P45 (P=0.7333). Histograms show mean±s.e.m. (d,e) Graphs represent the percentages of Sirt1+Ki67+ & NG2+Ki67+ cells after HX white matter in WT & HIF1α KO mice. Number in parentheses within bar indicates number of samples (n=4 brains per group & per genotype; ****P<0.0001, one-way analysis of variance, Bonferroni post hoc test, mean±s.e.m.). (f,g) Western blot demonstrates no increase (P=0.8231) in Sirt1 & HIF1α expression in white matter of Hif1α KO mice. Actin was used as loading control (mean±s.e.m.; n=3 brains for each experiment, group & genotype). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms13866), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



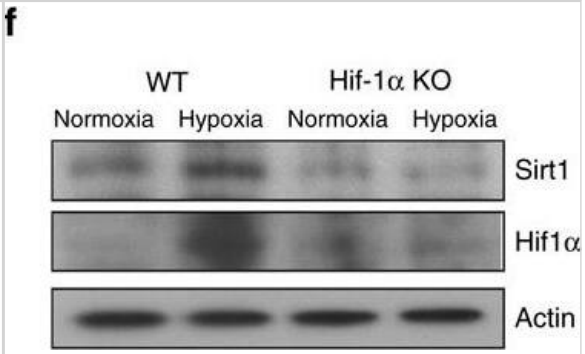
Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Results of in situ hybridization & immunohistochemistry on thin adjacent section to detect expression of HIF-1α1.2 mRNA & HIF1α protein in malignant & benign prostate tissue. In situ hybridization (antisense probe, Fig. 3a) & immunostaining with HIF1α Ab2 (Fig. 3c) on thin adjacent sections of NE-differentiated prostate adenocarcinoma showed co-localization of HIF1α1.2 transcript & HIF-1α protein. Incubation with sense probe did not generate any detectable hybridization signals (Fig. 3b). Both In situ hybridization (Fig. 3d antisense) & HIF-1α Ab2 immunostaining (Fig. 3f) were negative in non-NE-differentiated prostate adenocarcinoma. In situ hybridization with sense probe performed on non-NE-differentiated prostate cancer (Fig. 3e) was negative. In situ hybridizering on benign prostate tissue showed HIF1α1.2 transcript in NE-like cells of benign prostate tissue (Fig. 3g, □). The sense probe on thin adjacent section generated no signals (Fig. 3h,□). Furthermore, co-localization of HIF1α1.2 transcript (Fig. 3i,□,□,□) & HIF1α protein, detected with HIF1αAb3 (Fig. 3j,□,□,□) was also shown in NE-like cells of benign prostate tissue. Panels a, b, c, d, e, f, g, h, i, j: 40× objective. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/20663134), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HIF-1α expression in primary cortical neurons exposed to hypoxia/ischemia. A & A') Neurons were double-stained for HIF-1α & MAP2 in the presence of 5 & 25 mM glucose with & without hypoxia. HIF-1α expression in the somata was observed in cells with interneuron-like morphology after hypoxia. B) CoCl2 (0.3 mM) induced HIF-1α expression in cells with interneuron-like morphology. C) Quantification represents the increase in HIF-1α–ir staining (mean ± SD; 5-10 neurons quantified from each experiment, n = 3 experiments). D)In vivo brain slice shows a similar pattern of positive HIF-1α -ir in round soma (open arrow) & negative in neurons with pyramidal-like morphology (solid arrow) in the ipsilateral side. *p < 0.05, compared with normoxia (25 mM glucose), #p < 0.05, compared with hypoxia (25 mM glucose). Scale bars, 20 µm (A, A', B); 10 µm (D). Image collected & cropped by CiteAb from the following publication (https://actaneurocomms.biomedcentral.com/articles/10.1186/2051-5960-2-51), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - HX-induced Sirt1 expression in white matter OPCs requires HIF1α.(a) HIF1α stabilization of Sirt1 transcript expression in OPCs as revealed by representative RT–PCR represents higher level of Sirt1 mRNA in VHL cKO mice. GDPDH mRNA serves as a control. Mean±s.e.m., n=3 brains for each group. (b,c) Representative western blot demonstrates a transient increase of HIF1α expression in HX white matter at P18 with no significant effect at P11 (P=0.7955) & P45 (P=0.7333). Histograms show mean±s.e.m. (d,e) Graphs represent the percentages of Sirt1+Ki67+ & NG2+Ki67+ cells after HX white matter in WT & HIF1α KO mice. Number in parentheses within bar indicates number of samples (n=4 brains per group & per genotype; ****P<0.0001, one-way analysis of variance, Bonferroni post hoc test, mean±s.e.m.). (f,g) Western blot demonstrates no increase (P=0.8231) in Sirt1 & HIF1α expression in white matter of Hif1α KO mice. Actin was used as loading control (mean±s.e.m.; n=3 brains for each experiment, group & genotype). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms13866), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications


Mandl M, Depping R. ARNT is a potential direct HIF-1 target gene in human Hep3B hepatocellular carcinoma cells. Cancer Cell Int. 2017-08-31 [PMID: 28855849]

Nanduri J, Wang N, Wang Bl, Prabhakar Nr Lysine demethylase KDM6B regulates HIF-1 alpha mediated systemic and cellular responses to intermittent hypoxia Physiological genomics 2021-07-23 [PMID: 34297635]

Jenna Kerry, Erin J Specker, Morgan Mizzoni, Andrea Brumwell, Leslie Fell, Jenna Goodbrand, Michael N Rosen, James Uniacke Autophagy-dependent alternative splicing of ribosomal protein S24 produces a more stable isoform that aids in hypoxic cell survival. FEBS letters 2024-03-12 [PMID: 38281767]

Yeh JL, Kuo CH, Shih PW et al. Xanthine derivative KMUP-1 ameliorates retinopathy Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2023-07-03 [PMID: 37406513]

Kerry J, Specker E, Mizzoni M et al. Autophagy-dependent alternative splicing event produces a more stable ribosomal protein S24 isoform that aids in hypoxic cell survival bioRxiv 2023-09-26 (WB)

Bharti A, Urs AB, Kumar P. Significance of HIF-1? Expression and LOXL-2 Localization in Progression of Oral Squamous Cell Carcinoma Asian Pacific Journal of Cancer Prevention 2021-02-01 [PMID: 33639646] (Immunohistochemistry, Immunohistochemistry-Paraffin)

Li X, Ma TK, Wang M et al. YY1-induced upregulation of LncRNA-ARAP1-AS2 and ARAP1 promotes diabetic kidney fibrosis via aberrant glycolysis associated with EGFR/PKM2/HIF-1? pathway Frontiers in Pharmacology 2023-02-15 [PMID: 36874012]

Segura S, Stolnicu S, Boros M et al. mTOR Pathway Activation Assessed by Immunohistochemistry in Cervical Biopsies of HPV-associated Endocervical Adenocarcinomas (HPVA): Correlation With Silva Invasion Patterns Applied Immunohistochemistry & Molecular Morphology 2021-08-01 [PMID: 33587450] (Immunohistochemistry)

Kang HJ, Min BK, Choi WI et al. Pyruvate dehydrogenase kinase 1 and 2 deficiency reduces high-fat diet-induced hypertrophic obesity and inhibits the differentiation of preadipocytes into mature adipocytes Experimental & Molecular Medicine 2021-09-22 [PMID: 34552205] (Western Blot)

Guo Y, Zhou J, Li X et al. The Association of Suppressed Hypoxia-Inducible Factor-1 Transactivation of Angiogenesis With Defective Recovery From Cerebral Ischemic Injury in Aged Rats Frontiers in Aging Neuroscience 2021-02-26 [PMID: 33716719]

Heyer, V;Reina-San-Martin, B; Optimal AID expression and efficient immunoglobulin class switch recombination are dependent on the Hypoxia-Inducible Factor European journal of immunology 2023-05-04 [PMID: 37143384] (WB, Mouse)

Pang X, Wang Z, Yin D, Zhang Z. Overexpression of hypoxia-inducible factor prolyl- hydoxylase attenuated by HCG-induced vascular endothelial growth factor expression in luteal cells. Mol Med Rep 2015-05-16 [PMID: 25975603]


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

## Procedures

**Western Blot protocol for HIF-1 alpha Antibody (NB100-123)**

Western Blot Protocol

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

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

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

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

**Immunohistochemistry protocol for HIF-1 alpha Antibody (NB100-123)**

Please see:

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

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

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

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