Product Datasheet

HIF-2 alpha/EPAS1 Antibody - BSA Free

NB100-122

Unit Size: 0.1 ml

Store at -20 °C.

Reviews: 37  Publications: 743

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Updated 8/21/2023 v.20.1

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## Product Information

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at -20 °C.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>96.5 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>2034</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>EPAS1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Fish, Hamster, Primate, Rabbit, Reptile, Sheep</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Use in Mouse reported in scientific literature (PMID:33758176).</td>
</tr>
<tr>
<td>Specificity/Sensitivity</td>
<td>This HIF-2 alpha/EPAS1 Antibody is specific for HIF-2 alpha/EPAS, and does not cross-react with HIF-1 alpha.</td>
</tr>
<tr>
<td>Immunogen</td>
<td>This HIF-2 alpha/EPAS1 Antibody was developed against a peptide derived from the C-terminus of mouse/human HIF-2 alpha protein.</td>
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## Product Application Details

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
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<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP), Dual RNAscope ISH-IHC, Knockdown Validated, Knockout Validated</td>
</tr>
<tr>
<td>Recommended Dilutions</td>
<td>Western Blot 1 - 2 ug/mL, Simple Western 1:50, Flow Cytometry, ELISA 1:100 - 1:2000, Immunohistochemistry 1:100, Immunocytochemistry/Immunofluorescence 1:100, Immunoprecipitation 5 ug / 1 mg lysate, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen, Immunoblotting reported in scientific literature (PMID 28115701), In vitro assay reported in scientific literature (PMID 24998849), Gel Super Shift Assays reported in scientific literature (PMID 15184875), SDS-Page, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockout Validated reported in scientific literature (PMID 26861754), Knockdown Validated reported in scientific literature (PMID 31061092), Dual RNAscope ISH-IHC</td>
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<tr>
<td>Application Notes</td>
<td>In WB, this antibody recognizes a band at 118 kDa representing HIF-2 alpha. Simple Western reported by an internal validation. Separated by Size- Wes/Sally Sue/Peggy Sue, antibody dilution of 1:50. Apparent MW in kDa on Simple Western was 110kDa; matrix was 12-230 kDa.</td>
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</tbody>
</table>
WNT11 is induced by hypoxia or hypoxic mimetics in different cell types. Immunoblot analyses of HeLa cells under normal air or hypoxia for 24 hrs. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/srep21520) licensed under a CC-BY license.

HIF-1alpha is the predominant transcriptional regulator of WNT11 expression during hypoxia. EMSCs isolated from the indicated mouse genotypes were infected with lentivirus expressing GFP or Cre recombinase. Non-infected cells and GFP infected cells served as controls. Immunoblot analyses of EMSCs derived from the indicated genotypes treated with 0.1 mM DMOG for 24 hrs. Attenuated WNT11 expression in Hif-1alpha KO EMSCs (lenti-Cre infected Hif-1af/f). Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/srep21520) licensed under a CC-BY license.

Formalin-fixed paraffin-embedded tissue sections of human placenta were probed for HIF-2 alpha/EPAS1 mRNA (ACD RNAscope Probe, catalog #410598; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal (Novus Biologicals catalog # NB100-122) at 1:100 dilution with one-hour incubation at room temperature followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to trophoblastic cells.

Expression of hypoxia-inducible alpha subunits in normal and diseased lung tissue. HIF-2alpha expression are more evident in fibroblasts from idiopathic pulmonary fibrosis (d) than from lung tissue affected by other inflammatory conditions (i.e. chronic bronchitis, panel e) or normal lung tissue (f); as demonstrated by a higher proportion of positive fibroblasts (open arrows) than negative ones (solid black arrows). Image collected and cropped by CiteAb from the following publication (https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1100-4) licensed under a CC-BY license.
Image shows a specific band for HIF-2 alpha in 0.5 mg/mL of hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Immunoblot validation of HIF2A and PHD2 KO clones using HIF2A (#NB100-122; dilution: 1/300), PHD2 (#NB100-137; dilution: 1/500). To blot HIFs factor cells were first pre-treated for 5 h with CoCl2 300 uM before protein extraction, a condition that promotes HIF factor accumulation. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/s41467-018-06988-3) licensed under a CC-BY license.

HIF-2 alpha in human retinal and choroidal primary endothelia lysates using .. Image from verified customer review.

HIF-2 alpha in MDA-MB-231 cell lysate (overexpression and endogenous samples) using .. did not react to HIF-1 alpha overexpression. Image from verified customer review.
Induced WNT11 expression with tumor hypoxia and WNT11 regulates
tumor growth. Bevacizumab increased expression of HIF-1alpha and
HIF-2alpha and WNT11. First 10 lanes are control tumors, and the last
10 lanes are tumors from bevacizumab-treated animals. Lysates from
whole tissue and nuclei are indicated. Alpha -Tubulin, actin and lamin
A/C are loading controls. Image collected and cropped by CiteAb from
the following publication (http://www.nature.com/articles/srep21520)
licensed under a CC-BY license.

MDA-MB-231 cells were exposed to 20% or 1% O2 for 16 hours, and
chromatin immunoprecipitation (ChIP) was performed with the indicated
antibody (Ab). Primers flanking the HIF binding site were used for qPCR.
ChIP image submitted by a verified customer review.

Lane 1: Cobalt chloride treated COS7 nuclear extracts. Lane 2:
Untreated COS7 nuclear extracts.

786-O cells without or with VHL overexpression. Image from verified
customer review.
Analysis using the HRP conjugate of NB100-122. Detection of normoxic and hypoxic nuclear rat cell lysates.

![Image](https://example.com/analysis.png)

HIF-2 alpha immunoreactivity in human cardiac myocytes stained with NB100-122.

![Image](https://example.com/hif-2alpha.png)


Reduction of HIF-2alpha levels leads to protection in UV-triggered apoptosis, but not for apoptosis caused by glucose and serum starvation in 786-O cells. Parental 786-O or those either stably expressing wildtype VHLp19 or stably infected with a control vector (pSuperRetro) or a pool of two HIF-2alpha shRNAs vectors [21] were grown to confluence and lysed. Cell alpha-tubulin.

![Image](https://example.com/reduction.png)
<table>
<thead>
<tr>
<th>Publications</th>
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<tbody>
<tr>
<td>Hulea L, Gravel SP, Morita M et al. Translational and HIF-1a-Dependent Metabolic Reprogramming Underpin Metabolic Plasticity and Responses to Kinase Inhibitors and Biguanides. Cell Metab. 2018-09-17 [PMID: 30244971] (B/N)</td>
</tr>
<tr>
<td>Details: Dilution: 1:500</td>
</tr>
<tr>
<td>Sangam S, Sun X, Schwantes-An TH et al. SOX17 Deficiency Mediates Pulmonary Hypertension: At the Crossroads of Sex, Metabolism, and Genetics American journal of respiratory and critical care medicine 2023-03-13 [PMID: 36913491]</td>
</tr>
<tr>
<td>Colson A, Depoix CL, Lambert I et al. Specific HIF-2? (Hypoxia-Inducible Factor-2) Inhibitor PT2385 Mitigates Placental Dysfunction In Vitro and in a Rat Model of Preeclampsia (RUPP) Hypertension (Dallas, Tex. : 1979) 2023-05-01 [PMID: 36876500] (WB, Human)</td>
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<tr>
<td>Kubaichuk K, Kietzmann T USP10 Contributes to Colon Carcinogenesis via mTOR/S6K Mediated HIF-1? but Not HIF-2? Protein Synthesis Cells 2023-06-08 [PMID: 37371055] (WB, Human, Mouse)</td>
</tr>
<tr>
<td>Sun H, Pratt RE, Dzau VJ, Hodgkinson CP Neonatal and adult cardiac fibroblasts exhibit inherent differences in cardiac regenerative capacity The Journal of biological chemistry 2023-04-10 [PMID: 37044217] (WB, ICC/IF, Mouse)</td>
</tr>
<tr>
<td>Mayro B, Hoj JP, Cerda-Smith CG et al. ABL kinases regulate the stabilization of HIF-1? and MYC through CPSF1 Proceedings of the National Academy of Sciences of the United States of America 2023-04-18 [PMID: 37040401]</td>
</tr>
<tr>
<td>Leu T, Denda J, WrobelN A, Fandrey J Hypoxia-Inducible Factor-2alpha Affects the MEK/ERK Signaling Pathway via Primary Cilia in Connection with the Intraflagellar Transport Protein 88 Homolog Molecular and cellular biology 2023-04-19 [PMID: 37074220]</td>
</tr>
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More publications at [http://www.novusbio.com/NB100-122](http://www.novusbio.com/NB100-122)
Procedures

Western Blot protocol for HIF-2 alpha/EPAS1 Antibody (NB100-122)

General considerations for Western blot analysis of HIF-alpha proteins

1. HIF-2alpha is degraded under normoxic conditions and it is stabilized at O2 concentrations below 5% or with treatment using certain agents (CoCl2, DFO, etc.).

2. Positive and negative controls should always be run side by side in a Western blot to accurately identify the protein band upregulated in the hypoxic sample.


4. To accurately compare treated and untreated samples and to ensure equal loading of samples the expression of a loading control should be evaluated.
   (alpha Tubulin Antibody (DM1A): NB100-690)

5. The fully post-translationally modified form of HIF-2alpha is ~118 kDa, or larger.

6. HIF-2alpha may form a heterodimer with HIF-1beta. However, this is not typically seeing under denaturing conditions.

Western Blot Protocol

Materials

1x Laemmli Sample Buffer: 2% SDS, 2.5% 2-mercaptoethanol (bME), 25% glycerol, 0.01% bromophenol blue, 62.5 mM Tris HC, pH 6.8

1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3

1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol.

1X TBS

TBST (1X TBS with 0.1% Tween-20)

Blocking solution: TBST with 5% non-fat dry milk

Rabbit polyclonal anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (~1-2 ug/mL)

Methods

Whole-Cell Lysates

1. Load samples of treated and untreated cell lysates, 10-40 mg of total protein per lane on a 7.5% polyacrylamide gel (SDS-PAGE). Alternatively, gradient gels can be used for better resolution of lower molecular weight loading controls.

2. Resolve proteins by electrophoresis as required.

3. Transfer proteins to 0.45 mm PVDF membrane for 1 hour at 100V or equivalent.

4. Stain the blot using Ponceau S for 1-2 minutes to confirm efficient protein transfer onto the membrane.
5. Rinse the blot in distilled water to remove excess stain and mark the lanes and locations of molecular weight markers using a pencil.

6. Block the membrane using Blocking solution for 1 hour.

7. Dilute the rabbit anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (1-2 ug/ml) and incubate 1 hour at room temperature or overnight at 4oC.

8. Wash the membrane 3X 10 min in TBST.

9. Incubate in the appropriate diluted rabbit-IgG HRP-conjugated secondary antibody in blocking solution (as per manufacturer's instructions) for 1 hour at room temperature.

10. Wash the membrane 3X10 min in TBST.

11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (e.g., ECL, ECL Plus). Image blot.

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**Immunocytochemistry/Immunofluorescence protocol for HIF-2 alpha/EPAS1 Antibody (NB100-122)**

HIF-2 alpha/EPAS1 Antibody: https://www.novusbio.com/products/hif-2-alpha-epas1-antibody_nb100-122

**Immunocytochemistry Protocol**

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS.  Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS.  Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.
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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