Product Datasheet

HIF-2 alpha/EPAS1 Antibody

NB100-122

Unit Size: 0.1 ml

Store at -20 °C.

Reviews: 30  Publications: 585

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Updated 4/25/2019 v.20.1

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# Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at -20 °C.</td>
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<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
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<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
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<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
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<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>118 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
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<tbody>
<tr>
<td><strong>Gene ID</strong></td>
<td>2034</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>EPAS1</td>
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<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Fish, Hamster, Primate, Rabbit, Sheep</td>
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**Reactivity Notes**

- Reactivity with sheep reported in the scientific literature (PMID: 17307811).
- Hamster reactivity reported in scientific literature (PMID: 24997364 and 24997360). Rabbit reactivity reported in scientific literature (PMID: 19112498).

**Specificity/Sensitivity**

Specific for HIF-2 alpha/EPAS. Does not cross-react with HIF-1 alpha.

**Immunogen**

A peptide derived from the C-terminus of mouse/human HIF-2 alpha protein.

## Product Application Details

### Applications

- Western Blot
- Simple Western
- Chromatin Immunoprecipitation
- ELISA
- Flow Cytometry
- Gel Super Shift Assays
- Immunoblotting
- Immunocytochemistry/Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry-Frozen
- Immunohistochemistry-Paraffin
- Immunoprecipitation
- Knockout Validated

### Recommended Dilutions

- Western Blot 1.0 ug/mL - 2.0 ug/mL
- Simple Western 1:50
- Chromatin Immunoprecipitation 1:10 - 1:500
- 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
- Gel Super Shift Assays
- Knockout Validated

### Application Notes

- Use in Gel Shift Super Assay reported in scientific literature (PMID: 15184875).
- Use in Immunoblotting reported in scientific literature (PMID 28115701). In WB, this antibody recognizes a band at 118 kDa representing HIF-2 alpha. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.
- Knock Out Validation was reported in scientific literature (PMID: 26861754).
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF2 expression in HIF2 over-expressed HEK 293T cells. Image from verified customer review.

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF-2 alpha in human retinal and choroidal primary endothelia lysates using anti-HIF-2 alpha antibody. Image from verified customer review.


Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis using the HRP conjugate of NB100-122. Detection of normoxic and hypoxic nuclear rat cell lysates.
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF-2 alpha in MDA-MB-231 cell lysate (overexpression and endogenous samples) using anti-HIF-2 alpha antibody. HIF-2 alpha antibody did not react to HIF-1 alpha overexpression. Image from verified customer review.

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Lane 1: Cobalt chloride treated COS7 nuclear extracts. Lane 2: Untreated COS7 nuclear extracts.

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - 786-O cells without or with VHL overexpression. Image from verified customer review.

Immunocytochemistry/Immunofluorescence: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF-2 alpha antibody was tested in RCC4 cells at a 1:200 dilution against DyLight 488 (Green). Alpha tubulin and nuclei were counterstained against DyLight 568 (Red) and DAPI (Blue), respectively.
Chromatin Immunoprecipitation: HIF-2 alpha/EPAS1 Antibody [NB100-122] - MCF7 cells were exposed to 20% or 1% O2 for 16h, and ChIP assays were performed using IgG or antibodies against HIF-1α, HIF-2α, HIF-1β. Image from verified customer review.

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis on normoxic and hypoxic nuclear rat cell lysates.

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Human Glioma whole cell lysates. Image from verified customer review.

Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF-2 alpha immunoreactivity in human cardiac myocytes stained with NB100-122.
Simple Western: HIF-2 alpha/EPAS1 Antibody [NB100-122] - 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.


Immunoblotting: HIF-2 alpha/EPAS1 Antibody [NB100-122] - CAIX/ HIF-2 alpha/EPAS1 and other antibodies used for immunoblotting to analyze the hypoxia (Hx, 1% O2) or total protein extracts from HeLa cells grown in normoxia (Nx, 21% O2) conditions. Ferecatu I, Canal F, Fabbri L, Mazure NM, Bouton C, Golinelli-Cohen M-P (2018) Dysfunction in the mitochondrial Fe-S assembly machinery leads to formation of the chemoresistant truncated VDAC1 isoform without HIF-1 alpha activation. PLoS ONE 13(3): e0194782.
<table>
<thead>
<tr>
<th>Publication</th>
<th>Date</th>
<th>PMID</th>
<th>WB/ Mouse</th>
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<tbody>
<tr>
<td>Chachami, G; Stankovic-Valentin, N; Karagiota, A; Basagianni, A; Plessmann, U; Urlaub, H; Melchior, F; Simos, G;</td>
<td>Mar 29 2019</td>
<td>30926672</td>
<td>WB, Human</td>
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<tr>
<td>Hypoxia-induced changes in SUMO conjugation affect transcriptional regulation under low oxygen Mol. Cell Proteomics</td>
<td>12:00AM</td>
<td></td>
<td></td>
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<tr>
<td>Gkotinakou, IM; Befani, C; Simos, G; Liakos, P; ERK1/2 phosphorylates HIF-2 alpha and regulates its activity by controlling its CRM1-dependent nuclear shuttling J. Cell. Sci.</td>
<td>Apr 8 2019</td>
<td>30962349</td>
<td>WB, Human</td>
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<td>Chaurasia, SN; Kushwaha, G; Kulkarni, PP; Mallick, RL; Latheef, NA; Mishra, JK; Dash, D; Platelet HIF-2 alpha promotes thrombogenicity through PAI-1 synthesis and extracellular vesicle release Haematologica</td>
<td>Apr 19 2019</td>
<td>31004026</td>
<td>WB, Human</td>
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<tr>
<td>Anea C, Merloiu A, Fulton D et al. Immunohistochemistry of the circadian clock in mouse and human vascular tissues. VP</td>
<td>Jul 20 2018</td>
<td>30101218</td>
<td>(IHC, Mouse)</td>
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<tr>
<td>Schofield HK, Tandon M, Park M et al. Pancreatic HIF2 alpha stabilization leads to chronic pancreatitis and predisposes to mucinous cystic neoplasm Cellular and Molecular Gastroenterology and Hepatology</td>
<td>Nov 10 2017</td>
<td>29693047</td>
<td>(WB, Mouse)</td>
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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)
Western Blot Protocol Specific for HIF-2alpha 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 seen 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 mouse-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.
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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Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications

Products Related to NB100-122

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<th>Product Code</th>
<th>Description</th>
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<tr>
<td>NB800-PC26</td>
<td>COS-7 Nuclear Hypoxic Induced Cell Lysate</td>
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<tr>
<td>HAF008</td>
<td>Goat anti-Rabbit IgG Secondary Antibody [HRP (Horseradish Peroxidase)]</td>
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<tr>
<td>NB7156</td>
<td>Goat anti-Rabbit IgG (H+L) Secondary Antibody</td>
</tr>
<tr>
<td>NBP2-24891</td>
<td>Rabbit IgG Isotype Control</td>
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