

HIF-1 alpha

Protein function	Functions as a master transcriptional regulator of the adaptive response to hypoxia. Under such conditions, it can activate over 40 genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. (SwissProt: Q16665)
Expression	<ul style="list-style-type: none"> - Most cell lines (under hypoxic conditions) - Most normal tissues (under hypoxic conditions) - Highest levels in kidney and heart - Overexpressed in majority of human cancers <p>Under normoxic conditions, HIF-1 alpha is largely undetectable and hypoxia will need to be induced in most cell lines and tissues before testing. Induction is typically not required for cancerous tissues where hypoxic regions are common within the tumor microenvironment (PubMed: 1850121, 11689469).</p>
Location	Cytoplasmic in normoxic conditions, nuclear translocation in response to hypoxia (PubMed: 9822602).
Isoforms	Isoform 1: 93 kDa (predicted) Isoform 2: 83 kDa (predicted) Isoform 3: 96 kDa (predicted)
Modifications	S-nitrosylation, Phosphorylation, Sumoylation, Acetylation, Polyubiquitination, Hydroxylation.
Positive controls	<p>WB: Hypoxic samples such as HeLa-DFO treated whole cell lysate ab116322.</p> <p>For a stronger signal, HeLa-DFO treated nuclear extracts are recommended ab180880. The cell fractionation kit can also be purchased separately ab109719.</p> <p>IHC: Cancerous tissues such as Human ovarian carcinoma, breast carcinoma, colonic adenocarcinoma and squamous cell cervical carcinoma tissues. Normal kidney and heart tissues may also be positive for HIF-1 alpha (PubMed: 1850121).</p>
Negative controls	Most normal tissues or cells, other than kidney or heart, under normoxic conditions.
Treatments	CoCl ₂ or DFO to induce hypoxia (PubMed: 3217626). Proteasome inhibitors like MG132 can stabilise HIF-1 alpha (PubMed: 19347037)

Western Blot:

HIF-1 alpha is stabilised only at O₂ concentrations below 5%. Under normoxic conditions HIF-1 alpha has a short half-life and may be degraded within 5-8 minutes in both nuclear and cytoplasmic compartments (PubMed: [11454738](#)). Therefore, proper sample preparation is critical to aiding WB success. If care hasn't been taken with sample preparation no bands may be seen on your blot.

Here are a few tips to help ensure the best results in WB blotting:

- Prepare cell lysates as quickly as possible; wash with ice cold PBS (on ice) and scraping cells directly in lysis buffer (on ice)
- Supplement cell lysis buffer with sufficient protease and phosphatase inhibitors
- Hypoxic chambers may be used to incubate samples overnight at low oxygen pressure to induce HIF-1 alpha levels
- Overnight incubation at 4°C with the primary antibody can also help.

Check individual antibody data sheets for specific recommendations.

Note: It is not uncommon to detect bands other than at the predicted 93 kDa in western blot due to the different forms of HIF-1 alpha:

- 40-80 kDa - degraded HIF-1 alpha.
- 110-130 kDa - post translationally modified HIF-1 alpha
- ~200 kDa – heterodimer with HIF-1 beta

Immunohistochemistry:

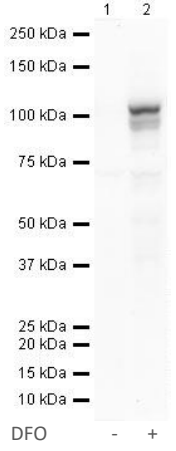
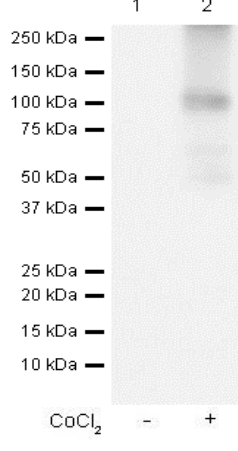
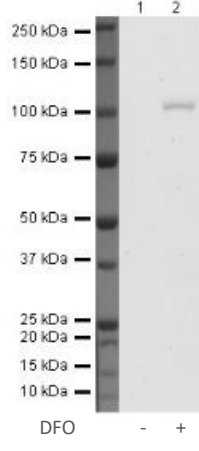
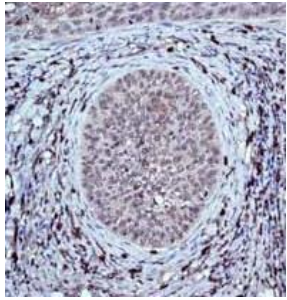
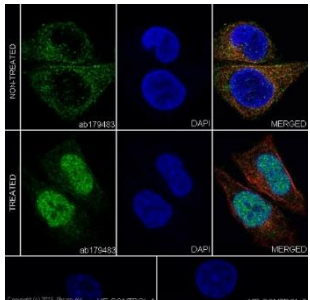
During hypoxia, HIF-1 alpha is stabilized and translocates to the nucleus to act as a transcription factor. You should see predominantly nuclear staining in your hypoxic samples but may observe some faint cytoplasmic staining.

- Fix tissue immediately after removal to prevent HIF-1 alpha degradation
- If you have difficulty detecting the target protein or your tissue sections are thicker than 20 µm, we recommend you **permeabilize** your samples.
- Perform heat mediated antigen retrieval (such as chamber/pressure cooker and microwave) to expose epitopes. Tris/EDTA pH 9.0 or citrate pH 6.0 are starting points.
- It is recommended to find a method that suits your particular tissue.

Check individual antibody data sheets for specific recommendations.

Note: Due to the inherent protein instability of HIF-1 alpha, we strongly recommend including a positive control when trying to detect this protein regardless of which antibody based application you are performing.

Product recommendations:

	Ab51608	Ab179483	Ab1
Clone	EP1215Y	EPR16897	H1alpha67
Host species	Rabbit	Rabbit	Mouse
Clonality	Monoclonal*	Monoclonal*	Monoclonal
Reacts with Species	Human	Mouse and human	Human, mouse, rat
Applications	Flow Cyt, ICC/IF, IHC-P, IP, WB	ICC/IF, WB	ICC/IF, IHC-P, IP, WB, Flow Cyt, CHIP
Validation data	 <p>Western blot analysis of Ab51608. Lane 1: DFO -, Lane 2: DFO +. Molecular weight markers (kDa): 250, 150, 100, 75, 50, 37, 25, 20, 15, 10.</p>	 <p>Western blot analysis of Ab179483. Lane 1: CoCl₂ -, Lane 2: CoCl₂ +. Molecular weight markers (kDa): 250, 150, 100, 75, 50, 37, 25, 20, 15, 10.</p>	 <p>Western blot analysis of Ab1. Lane 1: DFO -, Lane 2: DFO +. Molecular weight markers (kDa): 250, 150, 100, 75, 50, 37, 25, 20, 15, 10.</p>
			
Recommended usage	Preferred antibody for use in human	Preferred antibody for use in mouse	Preferred mouse monoclonal

	Ab51608	Ab179483	Ab1
Additional Information	ab51608 datasheet	ab179483 datasheet	ab1 datasheet

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