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Product datasheet

Human HIF-1 alpha ELISA Kit, Fluorescent ab229433

CatchPoint® SimpleStep ELISA®

5 Images

Overview

Product name Human HIF-1 alpha ELISA Kit, Fluorescent

Detection method Fluorescent

Precision

Intra-assay

Sample	n	Mean	SD	CV%
DFO HeLa	5			4.3%

Inter-assay

Sample	n	Mean	SD	CV%
DFO HeLa	3			7%

Sample type Cell culture extracts

Assay type Sandwich (quantitative)

Sensitivity 12 pg/ml

Range 0.03 ng/ml - 120 ng/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture media	112	94% - 129%
Fetal Bovine Serum	109	102% - 120%
Bovine Serum Albumin	97	93% - 106%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Human

Product overview HIF-1 alpha *in vitro* CatchPoint® SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit

is designed for the quantitative measurement of HIF1a protein in human cell extracts.

This CatchPoint SimpleStep ELISA kit has been **optimized for Molecular Devices Microplate Readers**. Click here for a list of recommended Microplate Readers.
If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.

The CatchPoint® SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. CatchPoint® HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

Notes

Hypoxia-inducible factor 1-alpha (HIF1 alpha) is a constitutively expressed transcription factor that is degraded under normal oxygen tensions but stabilized when oxygen is limiting (hypoxia). Under hypoxic conditions, stabilized HIF1 alpha translocates to the nucleus and promotes the transcription of a host of genes that enable the cell to adapt to the lack of oxygen. Aspects of the HIF1 alpha mediated hypoxic response include promotion of angiogenesis and the switch from aerobic respiration to anaerobic glycolysis. Many of the HIF1 alpha responsive genes encode proteins that promote glycolysis and/or inhibit oxidative phosphorylation (known as the Warburg effect). An exciting and developing area of current cancer research is examining how HIF-mediated metabolic reprogramming promotes tumor growth and survival.

In most cases, HIF1 alpha will need to be stabilized to be measured (steady state levels of HIF1 alpha in non-hypoxic environments is exceeding low in most cell lines). This can be achieved by (a) creating a hypoxic environment (e.g. using a hypoxia chamber) or (b) by using chemical treatments that mimic hypoxia (e.g. cobalt chloride or deferoxamine). The sample data in this assay protocol was generated using deferoxamine (DFO). DFO is an iron chelator and disrupts the function the prolyl hydroxylases that degrade HIF1 alpha in normoxia. By disrupting the enzymes that degrade HIF1 alpha, DFO increases the abundance of HIF1 alpha protein.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
100X Stoplight Red Substrate	1 x 120µl
10X Human HIF1a Capture Antibody	1 x 600µl
10X Human HIF1a Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
500X Hydrogen Peroxide (H_2O_2, 3%)	1 x 50µl
50X Cell Extraction Enhancer Solution	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 5B	1 x 6ml
HIF1a Human Lyophilized Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated Black 96-Well Microplate	1 unit
Stoplight Red Substrate Buffer	1 x 12ml

Function

Functions as a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions activates the transcription of over 40 genes, including, erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. Plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. Binds to core DNA sequence 5'-[AG]CGTG-3' within the hypoxia response element (HRE) of target gene promoters. Activation requires recruitment of transcriptional coactivators such as CREBPB and EP300. Activity is enhanced by interaction with both, NCOA1 or NCOA2. Interaction with redox regulatory protein APEX seems to activate CTAD and potentiates activation by NCOA1 and CREBBP.

Tissue specificity

Expressed in most tissues with highest levels in kidney and heart. Overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia and as a result of mutations in genes encoding oncoproteins and tumor suppressors.

Sequence similarities

Contains 1 basic helix-loop-helix (bHLH) domain.
Contains 1 PAC (PAS-associated C-terminal) domain.
Contains 2 PAS (PER-ARNT-SIM) domains.

Domain

Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).

Post-translational modifications

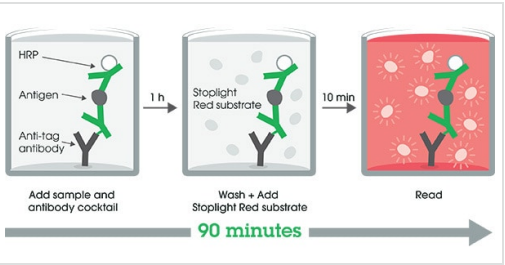
In normoxia, is hydroxylated on Pro-402 and Pro-564 in the oxygen-dependent degradation domain (ODD) by EGLN1/PHD1 and EGLN2/PHD2. EGLN3/PHD3 has also been shown to hydroxylate Pro-564. The hydroxylated prolines promote interaction with VHL, initiating rapid ubiquitination and subsequent proteasomal degradation. Deubiquitinated by USP20. Under hypoxia, proline hydroxylation is impaired and ubiquitination is attenuated, resulting in

stabilization.
In normoxia, is hydroxylated on Asn-803 by HIF1AN, thus abrogating interaction with CREBBP and EP300 and preventing transcriptional activation. This hydroxylation is inhibited by the Cu/Zn-chelator, Clioquinol.
S-nitrosylation of Cys-800 may be responsible for increased recruitment of p300 coactivator necessary for transcriptional activity of HIF-1 complex.
Requires phosphorylation for DNA-binding.
Sumoylated; by SUMO1 under hypoxia. Sumoylation is enhanced through interaction with RWDD3. Desumoylation by SENP1 leads to increased HIF1A stability and transriptional activity.
Ubiquitinated; in normoxia, following hydroxylation and interaction with VHL. Lys-532 appears to be the principal site of ubiquitination. Clioquinol, the Cu/Zn-chelator, inhibits ubiquitination through preventing hydroxylation at Asn-803.
The iron and 2-oxoglutarate dependent 3-hydroxylation of asparagine is (S) stereospecific within HIF CTAD domains.

Cellular localization

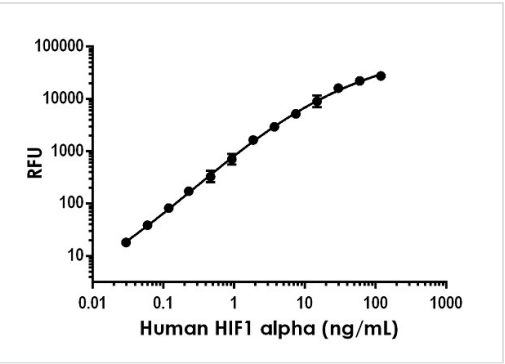
Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

Images



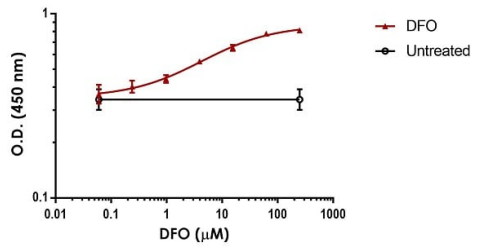
Other - Human HIF-1 alpha ELISA Kit, Fluorescent (ab229433)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



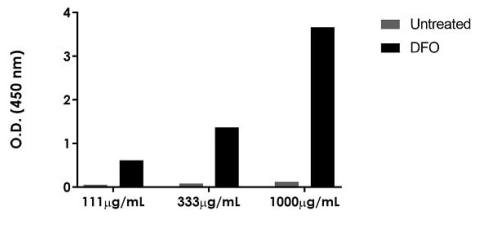
Example of human HIF1a standard curve in 1X Cell Extraction Buffer PTR.

The HIF1a standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



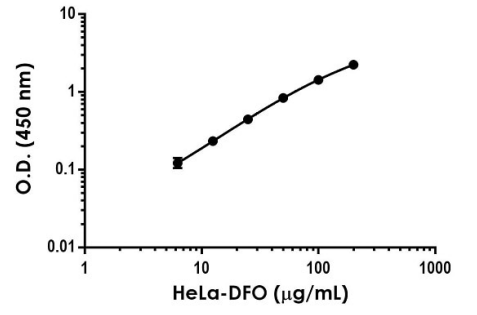
Dose-dependent induction of HIF1 alpha in HeLa cells by deferoxamine (DFO).

HeLa cells were cultured in 96-well tissue culture plates and were either untreated or exposed to varying dose of DFO for 24 hours. Cells were extracted directly in the culture plate by overlaying culture media with Extraction Buffer PTR (with Extraction Enhancer) such that the final concentration was 1X Extraction Buffer. Extracts were applied to the HIF1 alpha ELISA. Raw data with standard deviation is plotted from triplicate measurements.



Comparison of HIF1 alpha expression in HeLa cell extracts (with and without DFO treatment).

Background subtracted OD450 nm data from three loading concentrations are shown. In the HeLa cell line, DFO treatment is required to detect HIF1 alpha protein.



Titration of HeLa-DFO extract within the working range of the assay.

Background subtracted data from duplicate measurements are plotted. To induce HIF1 alpha protein levels, HeLa cells were treated with 500 µM Deferoxamine (DFO) for 24 hours.

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