abcam

Product datasheet

HIF-1 alpha Transcription Factor Assay Kit ab133104

21 References 4 Images

Overview

Product name	HIF-1 alpha Transcription Factor Assay Kit
Detection method	Colorimetric
Sample type	Cell culture extracts, Cell Lysate
Assay type	Semi-quantitative
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Mammals
Product overview	HIF-1 alpha Transcription Factor Assay (ab133104) is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysate. A 96-well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the HIF-1 alpha response element (5'-ACGTG-3') is immobilized to the wells of a 96-well plate. HIF-1 alpha contained in a nuclear extract, binds specifically to the HIF-1 alpha response element. The HIF transcription factor complex is detected by addition of a specific primary antibody directed against HIF-1 alpha. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.
Platform	Microplate reader

Properties

Storage instructions	Please refer to protocols.

Components	96 tests
96-Well Plate Cover	1 unit
Polysorbate 20	1 vial
Transcription Factor Antibody Binding Buffer (10X)	1 x 3ml
Transcription Factor Binding Assay Buffer (4X)	1 x 3ml

Components	96 tests
Transcription Factor Developing Solution	1 x 12ml
Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 x 100µl
Transcription Factor HIF-1 alpha 96-Well Strip Plate	1 unit
Transcription Factor HIF-1 alpha Competitor dsDNA	1 vial
Transcription Factor HIF-1 alpha Positive Control	1 vial
Transcription Factor HIF-1 alpha Primary Antibody	1 vial
Transcription Factor Reagent A	1 x 120µl
Transcription Factor Stop Solution	1 x 12ml
Wash Buffer Concentrate (400X)	1 x 5ml

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).

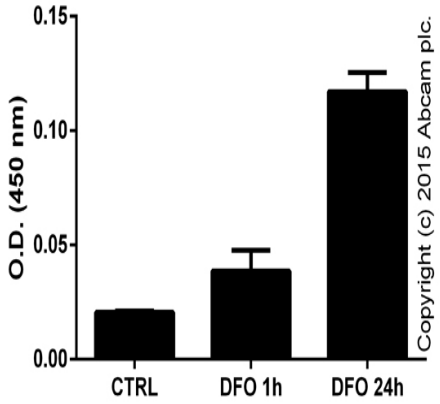
Post-translational modifications

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.

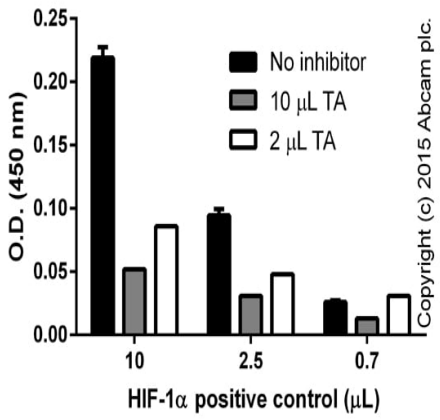
Cellular localization Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

Images



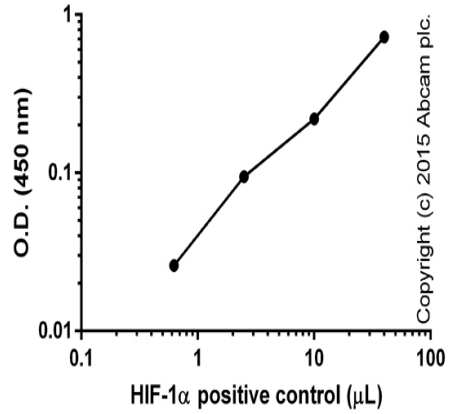
Functional Assay: ab133104 HIF-1 alpha Transcription Factor Assay Kit

HEK293 cells were treated with 1 mM deferoxamine mesylate (DFO) for 1 or 24 hours. 40 micoliters of nuclear lysates (**ab113474**; corresponding to 4e6 cells) were tested in duplicates (+/- SD).



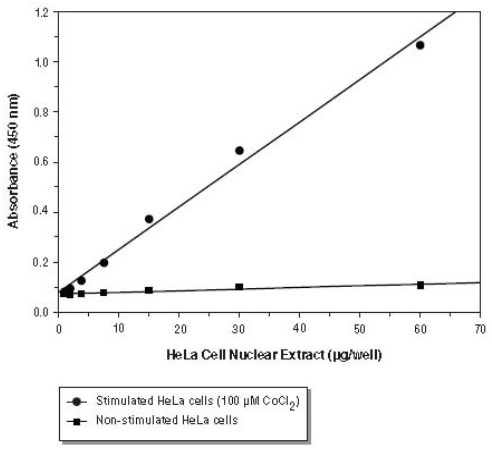
Functional Assay: ab133104 HIF-1 alpha Transcription Factor Assay Kit

Titration of positive control with different volumes of inhibitor (TA), background signal subtracted (duplicates; +/- SD).



Titration of positive control, background signal subtracted (duplicates; +/- SD).

Functional Assay: ab133104 HIF-1 alpha Transcription Factor Assay Kit



Assay of nuclear extract from stimulated HeLa cells (100 µM $CoCl_2$) and non-stimulated HeLa cells.

Functional Studies - HIF-1 alpha Transcription Factor Assay Kit (ab133104)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors