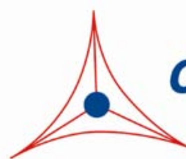
Product Manual

HIF- 1 Alpha Sandwich ELISA Kit

Catalog Number

CBA- 280 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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Introduction

Mammalian cells are able to sense low oxygen conditions and turn on a series of genes in response to the lack of oxygen. The hypoxia-inducible factor 1 transcriptional activator complex (HIF-1) is involved in the activation of several hypoxia-responsive genes including erythropoietin and VEGF. The HIF-1 complex is composed of two protein subunits: the constitutively expressed HIF-1b/ARNT (aryl hydrocarbon receptor nuclear translocator), and HIF-1 Alpha, the latter of which is not detected during normoxia since it is continually degraded by the ubiquitin proteasome system (UPS). In the presence of low oxygen conditions, however, HIF-1 Alpha is stabilized, accumulates, translocates from the cytosol to the nucleus, dimerizes with HIF-1b/ARNT, and becomes transcriptionally active. The activated HIF-1 complex then associates with hypoxic response elements (HREs) and binds transcriptional coactivators to induce gene expression. Tight regulation of the stability and function of HIF-1 is controlled by its post-translational modifications, such as hydroxylation, ubiquitination, acetylation, and phosphorylation.

Under normal oxygen conditions, the post-translational modification of HIF-1 Alpha occurs within several domains such as the N-terminal transactivation domain, C-terminal transactivation domain, and oxygen dependent degradation domain (ODDD). Hydroxylation of two proline residues and acetylation of a lysine residue in its ODDD promote binding of HIF-1 with the von Hippel-Lindau (pVHL) ubiquitin E3 ligase complex. This pVHL complex modifies HIF-1 with ubiquitin, marking it for degradation by the 26S proteasome. Furthermore, hydroxylation of C-terminal asparagine residue in the c-terminal transactivation domain blocks association of HIF-1 with CBP/p300 and as a result inhibits HIF-1 transcriptional activity. Upon synthesis of HIF-1 Alpha, the protein is rapidly hydroxylated by a family of 2-oxoglutarate dioxygenases on proline 402 and 564. Hypoxic or chemical inactivation of these dioxygenases (which were later termed proline hydroxylase domains (PHDs)), leads to an increase in the half life of HIF-1 Alpha and subsequent activation of HIF-1 complex.

Cell Biolabs' HIF-1 Alpha Sandwich ELISA Kit is an immunoassay developed for rapid detection of HIF-1 Alpha in any protein sample. HIF-1 Alpha protein is captured by a mouse monoclonal antibody that is attached to the well. HIF-1 Alpha is then detected with a goat polyclonal anti-HIF-1 Alpha antibody followed by an HRP conjugated secondary antibody. Each kit provides sufficient reagents to perform up to a total of 96 assays, and can detect HIF-1 Alpha from human, mouse, or rat.

Related Products

1. CBA-281: HIF-1 Alpha Cell Based ELISA
2. CBA-282: HIF-1 Alpha DNA Binding Activity Assay Kit
3. STA-320: Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-321: DNA Double-Strand Break (DSB) Staining Kit
5. STA-322: UV-induced DNA Damage ELISA Kit (CPD Quantitation)
6. STA-323: UV-induced DNA Damage ELISA Kit (6-4PP Quantitation)
7. STA-324: Oxidative DNA Damage Quantitation Kit (AP sites)
8. STA-325: Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)

9. STA-351: Comet Assay Kit (3-Well Slides), 75 Assays
10. STA-355: 96-Well Comet Assay Kit
11. STA-357: BPDE DNA Adduct ELISA Kit
12. STA-380: Global DNA Methylation ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HIF-1 Alpha Antibody Coated Plate (Part No. 128001): One strip well 96-well plate.
2. Anti-HIF-1 Alpha Antibody (Part No. 128002): One 20 µL vial of anti-HIF-1 Alpha Antibody.
3. Secondary Antibody, HRP Conjugate (Part No. 231704): One 20 µL vial.
4. Nuclear Extract Dilution Buffer (NEDB) (Part No. 128003): One 50 mL bottle.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. HIF-1 Alpha Standard (Part No. 128004): One 40 µL vial of 10 µg/mL HIF-1 alpha in Assay Diluent.

Materials Not Supplied

1. Nuclear extracts, whole cell lysates or tissue homogenates prepared according to the Preparation or Samples section below
2. PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store the HIF-1 Alpha Standard at -80ºC and the Anti-HIF-1 Alpha Antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

- Anti-HIF-1 Alpha Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the Anti-HIF-1 Alpha Antibody 1:500 with Assay Diluent. Immediately before use dilute the Secondary Antibody HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of HIF-1 Alpha Standard

Prepare a dilution series of HIF-1 Alpha standards in the concentration range of 0 to 80 ng/mL in Nuclear Extract Dilution Buffer (NEDB) according to Table 1 below.

Standard Tubes	10 µg/mL HIF-1 Alpha Standard (µL)	NEDB (µL)	HIF-1 Alpha (ng/mL)
1	8	992	80
2	500 of Tube #1	500	40
3	500 of Tube #2	500	20
4	500 of Tube #3	500	10
5	500 of Tube #4	500	5
6	500 of Tube #5	500	2.5
7	500 of Tube #6	500	1.25
8	0	500	0

Table 1. Preparation of HIF-1 Alpha Standards.

Preparation of Samples

Note: RIPA buffer inhibits this assay.

- Whole Cell Lysates or Tissue Homogenates: Prepare using the following lysis buffer: 50 mM Tris (pH 7.4), 300 mM NaCl, 10% (w/v) glycerol,3 mM EDTA, 1 mM MgCl2, 25 mM NaF, 20 mM-glycerophosphate, 1% Triton X-100, 25 µg/mL Leupeptin, 25 µg/mL Pepstatin, and 3 µg/mL Aprotinin.

- Nuclear Extracts: Prepare using Cell Biolabs' Nuclear/Cytosolic Fractionation Kit (Cat. #AKR-171) or other desired method.

Assay Protocol

1. Prepare any dilutions of unknown samples in the provided Nuclear Extract Dilution Buffer (NEDB).
2. Add 100 µL of HIF-1 Alpha unknown sample or standard to the Anti-HIF-1 Alpha Antibody Coated Plate. Each HIF-1 Alpha unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.

4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After each wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted anti-HIF-1 Alpha Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
7. Add 100 µL of the diluted Secondary Antibody HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical HIF-1 Alpha Sandwich ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

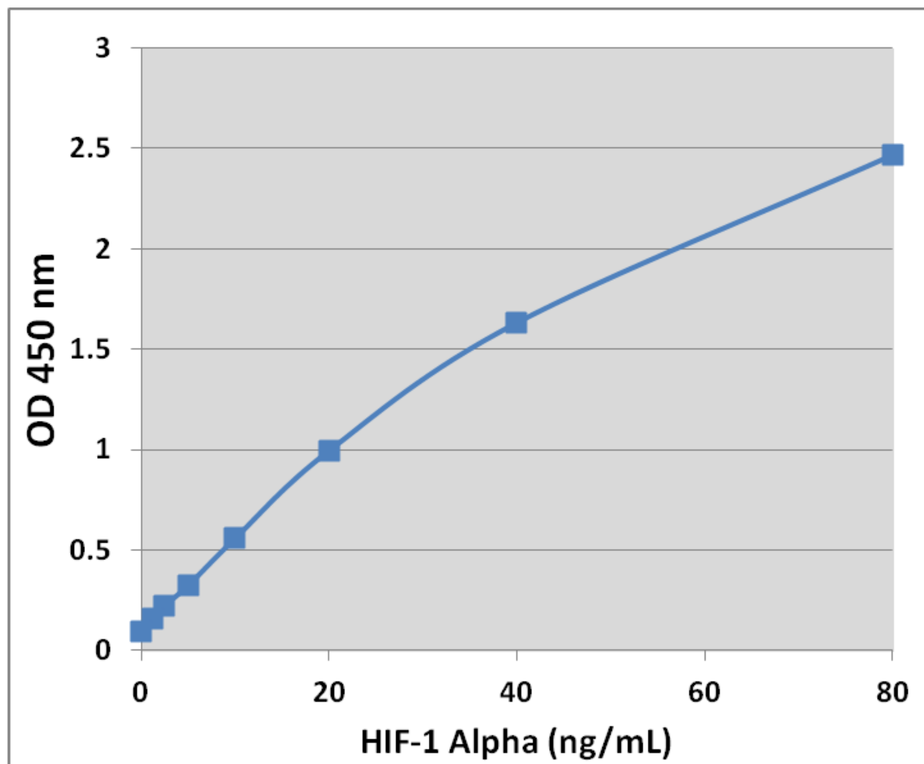

Figure 1: HIF-1 Alpha Standard Curve

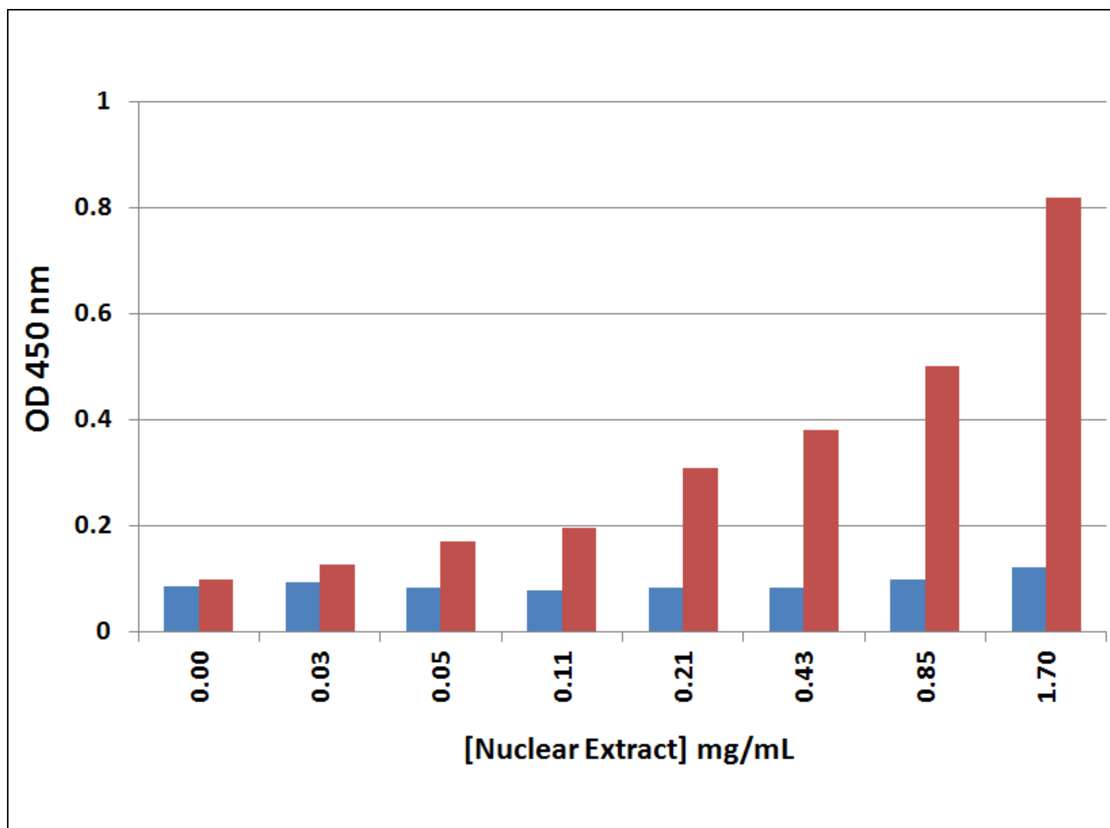

Figure 2: Demonstration of Nuclear HIF-1 Alpha Detection. Nearly confluent HeLa cells were incubated in the presence or absence of 0.2 mM Deferoxamine Mesylate (DFO) for 4 hours at 37ºC. Cells were then trypsinized from the plate, and nuclear extracts were prepared using Cell Biolabs' Nuclear/Cytosolic Fractionation Kit (Cat. #AKR-171). Nuclear extract from untreated HeLa cells (blue bars) or DFO treated HeLa cells (red bars) were measured for HIF-1 alpha levels according to the Assay Protocol.

References

1. Ke Q and Costa M. (2006) *Molecular Pharm.* **70**:1469-1480.
2. Salceda S and Caro J (1997) *J. Biol. Chem.* **272**: 22642-22647.
3. CW Pugh, JF O'Rourke, M Nagao, JM Gleadle, and PJ Ratcliffe (1997) *J. Biol. Chem.* **272**: 11205-11214.
4. LE Huang, Z Arany, DM Livingston, and HF Bunn (1996) *J. Biol. Chem.* **272**: 32253-32259.
5. PJ Kallio, I Pongratz, K Gradin, J McGuire, and L Poellinger (1997) *Proc Natl Acad Sci USA* **94**: 5667-5672**.**

Recent Product Citation

Ohyama, A. et al. (2016). Establishment and characterization of a clear cell carcinoma cell line, designated NOCC, derived from human ovary. *Human Cell*. doi:10.1007/s13577-016-0142-x.

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Warranty

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