Product Manual

HIF- 1 Alpha Cell Based ELISA Kit

Catalog Number

CBA- 281 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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. 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: hydroxylation of two proline residues and acetylation of a lysine residue in its oxygen dependent degradation domain (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 Cell Based ELISA Kit is an immunoassay developed for rapid detection of HIF-1 Alpha in fixed cells. Cells on a microplate are stimulated for HIF-1 Alpha stabilization, fixed, permeabilized, and then neutralized in the well. HIF-1 Alpha is then detected with an 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-280: HIF-1 Alpha Sandwich 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

1. Anti-HIF-1 Alpha Antibody (Part No. 128101): One 10 µL vial of anti-HIF-1 Alpha.
2. Secondary Antibody, HRP Conjugate (Part No. 231704): One 20 µL vial.
3. 10X Wash Buffer (Part No. 128102): One 30 mL bottle.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. Chemiluminescent Reagent A (Part No. 250102): One 6 mL amber bottle.
6. Chemiluminescent Reagent B (Part. No. 250103): One 6 mL clear bottle

Materials Not Supplied

1. Adherent cells
2. 37% Formaldehyde
3. Hydrogen Peroxide
4. Sodium Azide
5. Phosphate Buffered Saline (PBS)
6. Plate sealing tape or adhesive covers
7. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
8. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
9. Multichannel micropipette reservoir
10. Shaking platform
11. Microplate reader capable of reading luminescence
12. 96-well white-walled cell culture plate (black-walled plate is an acceptable but less desirable alternative)

Storage

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

Preparation of Reagents

- Fixation Buffer: Immediately before use, dilute 37% Formaldeyde to 4% (for adherent cells) in PBS.
- 1X Wash Buffer: Dilute the 10X Wash Buffer with deionized water to 1X. Stir to homogeneity.

- Anti-HIF-1 Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the Anti-HIF-1 alpha Antibody 1:2000 with Assay Diluent. Immediately before use dilute the Secondary Antibody HRP Conjugate 1:2000 with Assay Diluent. Do not store diluted solutions.
- Neutralizing Buffer: Immediately before use, add hydrogen peroxide and sodium azide to 1X Wash Buffer at a final concentration of 1% and 0.1%, respectively.
- Chemiluminescent Reagent: Immediately before use, mix equal volumes of Chemiluminescent Reagent A with Chemiluminescent Reagent B. Do not store diluted solutions.

Assay Protocol

1. Plate adherent cells in a 96-well white-walled (preferred) or black-walled cell culture plate so that they reach 70-80% confluency by the time they are treated or fixed.
2. Perform cell treatments as desired.

   Note: Perform all aspiration and addition steps below slowly and carefully with a manual multichannel pipette to minimize cell loss during the procedure.
3. Manually aspirate the wells with a multichannel pipette and replace media with 100 µL of Fixation Buffer. Seal the wells with an adhesive cover and incubate for 20 minutes at room temperature. *Note: if an adhesive cover is not available, seal in a zip lock bag or incubate uncovered in a fume hood.*
4. Remove Fixation Buffer and wash 3 times with 200 µL 1X Wash Buffer. Incubate each wash 5 minutes with gentle shaking on a platform shaker.
5. Remove 1X Wash Buffer and add 100 µL of Neutralizing Buffer and incubate 20 minutes at room temperature on bench.
6. Remove Neutralizing Buffer and wash 2 times with 200 µL 1X Wash Buffer. Incubate each wash 5 minutes with gentle shaking on a platform shaker.
7. Remove 1X Wash Buffer and add 200 µL Assay Diluent. Incubate for 1 hour at room temperature on the benchtop.
8. Aspirate wells and add 100 µL of the diluted anti-HIF-1 Alpha Antibody to each well. Incubate overnight at 4 deg C.
9. Wash the strip wells 3 times with 200 µL 1X Wash Buffer.
10. Add 100 µL of the diluted Secondary Antibody HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
11. Wash the strip wells 3 times with 200 µL PBS.

12. Add 100 µL of Chemiluminescent Reagent (see Preparation of Reagents Section) to each well, including the blank wells. Incubate at room temperature for 5 minutes on an orbital shaker.
13. Read the luminescence of each microwell on a plate luminometer.

Example of Results

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

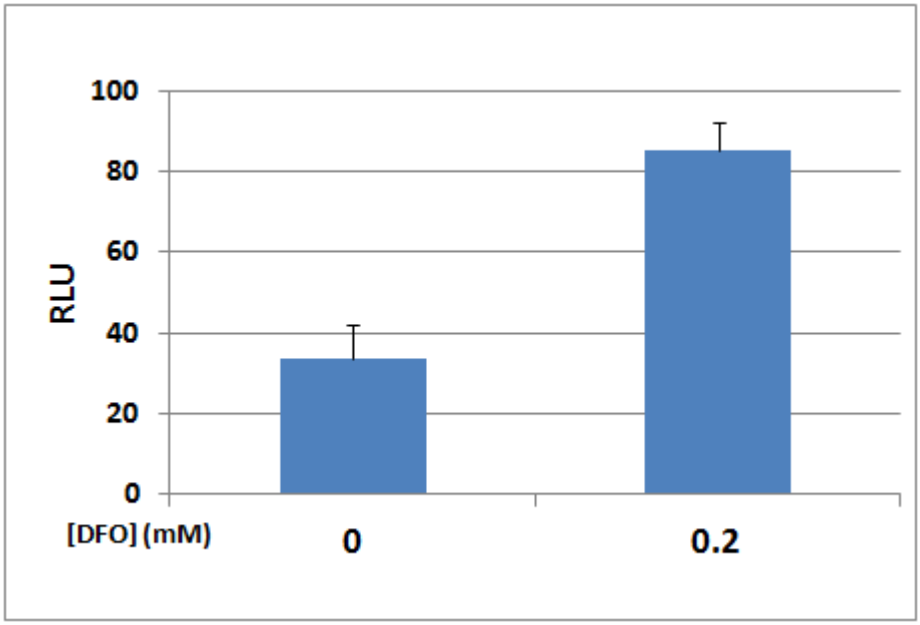

Figure 1: Demonstration of detection specificity of HIF-1 Alpha. Nearly confluent HeLa cells were incubated in the presence or absence of 0.2 mM Deferoxamine Mesylate (DFO) for 4 hours at 37ºC. Cells were then fixed and processed according to the Assay Protocol.

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**.**

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com