Product Manual

HIF- 1 Alpha DNA Binding Activity Assay Kit

Catalog Number

CBA- 282 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 Alpha DNA Binding Activity Assay Kit is an immunoassay developed for rapid detection of activated HIF-1 in any protein sample. Active HIF-1 complex is captured on a double stranded oligonucleotide containing an HRE that is attached to 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. The HIF-1 DNA Binding Activity Assay takes only 4.5 hours to complete compared to a standard 3 day electromobility shift (EMSA) method.

Assay Principle

The HIF-1 Alpha DNA Binding Activity Assay Kit is an ELISA for the measurement of activated HIF-1. A biotinylated double stranded DNA containing an HRE is first bound to the provided Streptavidin plate wells. Then protein samples are added to the DNA conjugated microplate. After a brief incubation, an anti-HIF-1 Alpha antibody is added, followed by an HRP conjugated secondary antibody, allowing for simple colorimetric detection by plate spectophotometry.

Related Products

1. CBA-280: HIF-1 Alpha Sandwich ELISA
2. CBA-281: HIF-1 Alpha Cell Based ELISA

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. 96-well Streptavidin Coated Plate (Part No. 128201): One strip well 96-well plate.
2. Biotinylated Double Stranded DNA (Part No. 128202): One 110 µL vial of 10 µM DNA containing a 50 mer double stranded oligonucleotide with 3 copies of the HRE.
3. Anti-HIF-1 Alpha Antibody (Part No. 128002): One 20 µL vial of antibody.
4. DNA Binding Buffer (Part No. 128203): One 10 mL bottle.
5. Heat Denatured Calf Thymus DNA (100X) (Part No. 128204): One 100 µL vial of 400 ng/mL DNA.
6. Secondary Antibody, HRP Conjugate (Part No. 231704): One 20 µL vial.
7. Assay Diluent (Part No. 310804): One 50 mL bottle.
8. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
9. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
10. Stop Solution (Part. No. 310808): One 12 mL bottle.

Materials Not Supplied

1. Nuclear extracts
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 Biotinylated Double Stranded DNA and 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.
- Complete DNA Binding Buffer: Dilute the Heat Denatured Calf Thymus DNA (100X) 1:100 into DNA Binding Buffer.

Preparation of Samples

Prepare nuclear extracts using Cell Biolabs' Nuclear/Cytosolic Fractionation Kit (Cat. #AKR-171) or by other desired nuclear extraction method.

Assay Protocol

1. Thaw the provided Biotinylated Double Stranded DNA, dilute 1:100 in PBS, and add 100 µL per well to the 96-well Streptavidin Coated Plate.
2. Incubate plate at room temperature on the bench top for one hour.
3. Wash wells 3 times with 250 µL of PBS 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 PBS.
4. Add 40 µL of Complete DNA Binding Buffer per well.
5. Add 10 µL of nuclear extract to each tested well. Each sample should be assayed in duplicate.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. 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.
8. 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.
9. Wash the strip wells 3 times according to step 7 above. Proceed immediately to the next step.

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. During this incubation, warm Substrate Solution to room temperature.
11. Wash the strip wells 3 times according to step 7 above. Proceed immediately to the next step.
12. 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.
13. 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).
14. 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 DNA Binding ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

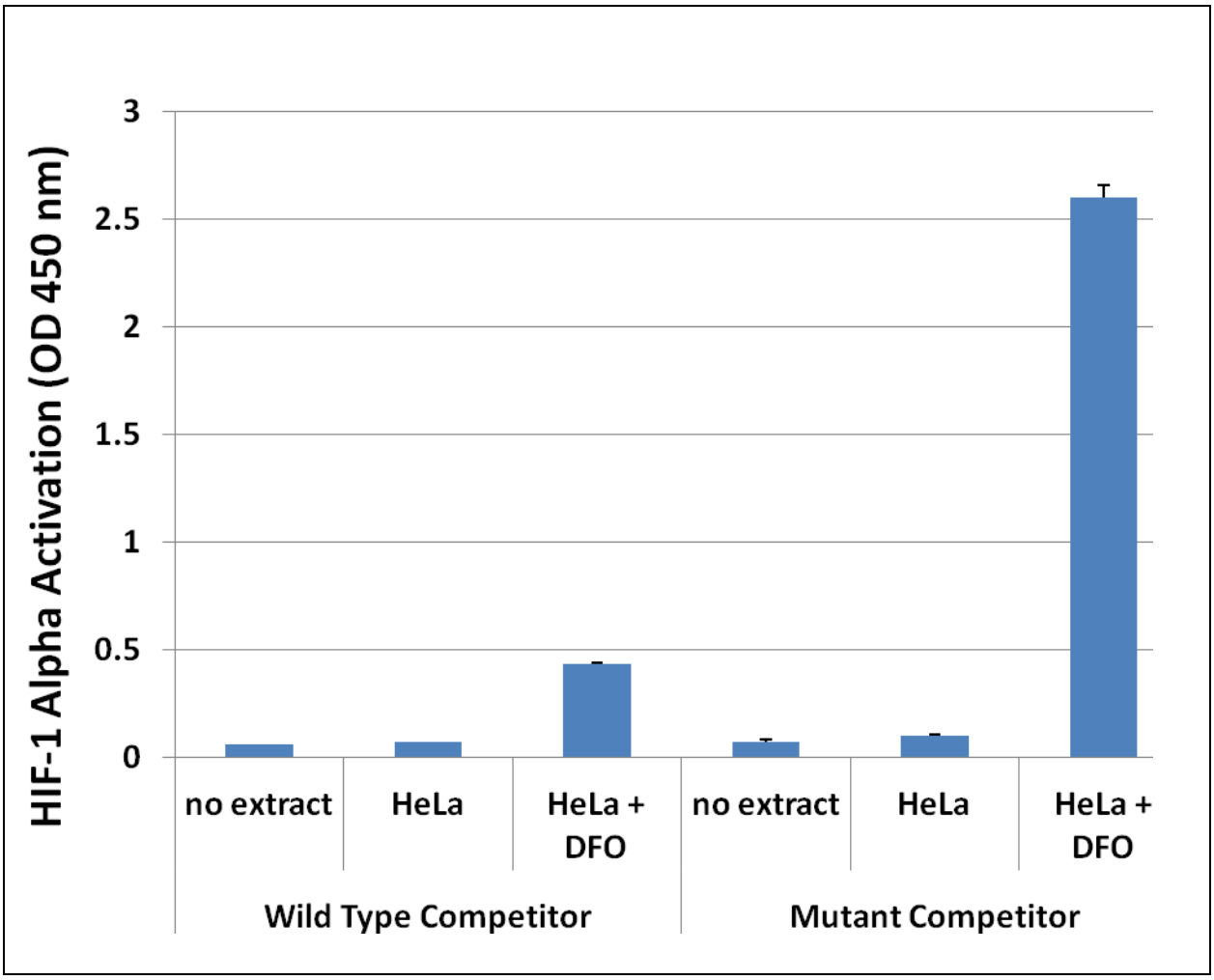

Figure 1: Demonstration of detection specificity of HIF-1 Alpha. Nearly confluent HeLa cells were incubated in the presence or absence of 0.2 mM Deferoxamine Mesylate (DFO) for 4 hours at 37ºC. Cells were then trypsinized from the plate, and nuclear extracts were prepared using Cell Biolabs' Nuclear/Cytosolic Fractionation Kit (Cat. #AKR-171). 100 pmol of non-biotinylated wild type or mutated hypoxic response element (HRE) double stranded competitor oligo (not provided) were added to the Complete DNA Binding Buffer just prior to addition to the well. 20 µg of nuclear extract was then added to each well and the ELISA was continued 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.

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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 exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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

©2014-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permission in writing.