HIF-1 alpha ELISA Kit

(For Human/Mouse)

Enzyme Immunoassay for the quantification of human/mouse HIF-1 alpha in cell lysates

Catalog number: ARG80969

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INTRODUCTION

Hif-1 alpha 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, HILPDA, 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. Involved in the axonal distribution and transport of mitochondria in neurons during hypoxia. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human HIF-1 alpha (HIF-1a) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any HIF-1a present is bound by the immobilized antibody. At the same time, a biotin-conjugated antibody specific for HIF-1a is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well

and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of HIF-1a bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of HIF-1a in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	96 well	4°C
Standards	8 ng/vial (2 vials)	4°C, Lyophilized
Sample Diluent	25 ml (2 vials)	4°C
Biotinylated HIF-1a antibody (36X)	1 vial	4°C
Antibody Diluent	12 ml	4°C
Streptavidin-HRP	1 vial	4°C
Streptavidin-HRP Diluent	12 ml	4°C
Chromogenic Solution A	6 ml	4°C (Protect from light)
Chromogenic Solution B	6 ml	4°C (Protect from light)
20X Wash buffer	50 ml	4°C
Plate sealer	1	RT
STOP solution	6 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell lysates- Wash cells twice gently with PBS. Scrap and collect cells in Lysis Buffer at the concentration of 1 X 10^7 cells/ml. Allow samples to sit on ice for

15 minutes. Centrifuge samples at 2000 x g for 5 minutes. Collect supernatant and perform assay as according to instructions. Samples may be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw.

Lysis buffer composition: 50mM Tris pH7.4, 300mM NaCl, 10% glycerol, 3mM EDTA, 1mM $MgCl_2$, 20mM glycerophosphate, 1% Triton X-100, Protease inhibitor.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Biotinylated HIF-1a antibody: Dilute 36X concentrated antibody in antibody diluent buffer. Allow to sit on ice for at least 1-2 hours before use. The solution should be prepared fresh. (Note: the volume of working solution per well is 0.1 ml)
- Standards: The standard solution should be prepared fresh. Reconstitute lyophilized vial (8ng/vial) with 1000 µl Sample Diluent Buffer to generate 800 ng/ml stock standard. Make 2-fold serial dilutions to generate standard concentration of 4000, 2000, 1000, 500, 250, and 125 pg/ml. Make sure that each tube has ≥ 300 µl of standard.
- Streptavidin-HRP working solution: The Streptavidin-HRP working solution should be prepared 1 hour prior to use. Streptavidin-HRP should be diluted 1:100 with Streptavidin-HRP Diluent buffer and mixed thoroughly. (Note: the volume of working solution per well is 0.1 ml)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl sample diluent buffer in duplicate into wells (blank).
3. Add 100 µl of standards and samples in duplicate into wells.
4. Cover with plate sealer and incubate the plate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of Biotinylated HIF-1a antibody to each well. Cover wells and incubate for 2 hours at room temperature.
7. Aspirate each well and wash as step 5.
8. Add 100 µl of Streptavidin-HRP solution in each well. Cover wells and incubate for 20 minutes at room temperature. Avoid exposure to direct light.
9. Aspirate each well and wash as step 5.
10. Add 50 µl of Chromogenic solution A and 50 µl of Chromogenic solution B subsequently to each well. Incubate for 10-15 minutes at room

temperature in dark.

11. Add 50 µl of Stop Solution to each well. Mix well.
12. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

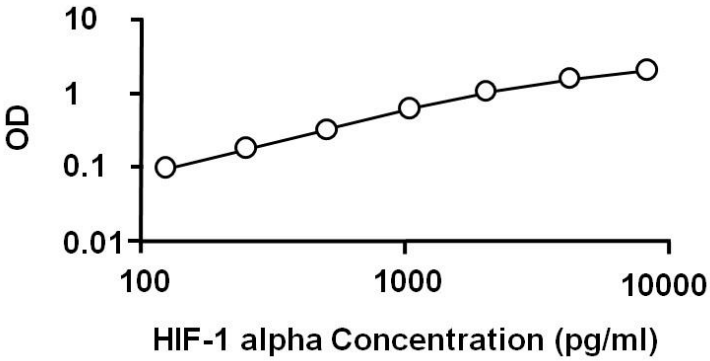
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of HIF-1 alpha ranged from 125-8000 pg/ml. The mean MDD was 100 pg/ml.