INSTRUCTIONS

Human HIF-1A ELISA Reagent Kit

ESSHIF1A

2545.1

Number	Description
ESSHIF1A	**Human HIF-1A ELISA Reagent Kit,** pre-titered coating and detection antibodies, recommended buffers and specific assay protocol optimized for the quantitative measurement of HIF-1A in human cell culture supernatants and serum.

Kit provides sufficient reagents for approximately five 96-well plates, provided the HIF-1A ELISA Reagent Kit Protocol is followed.

Kit Contents	Size	Assay Dilution
HIF-1A Coating Antibody	0.625mL	1:100
Lyophilized Recombinant HIF-1A Standard	5 vials	See vial label
HIF-1A Detection Antibody	0.625mL	1:100
Streptavidin-HRP	0.25mL	1:400
Substrate Solution	55mL	Ready to use
Stop Solution, 0.16M Sulfuric Acid	55mL	Ready to use

For research use only. Not for use in diagnostic procedures.

Storage: Immediately upon receipt, aliquot and freeze the coating and detecting antibodies at ≤ -20°C in a manual defrost freezer (125µL/tube). Avoid repeated freeze-thaw cycles. Store all other components at 2-8ºC. Kit is shipped on dry ice.

Table of Contents

Introduction

The Thermo Scientific™ Human HIF-1 Alpha ELISA Kit measures human hypoxia-inducible factor 1-alpha (HIF1A) protein in serum, plasma and cell culture supernatants using the quantitative sandwich ELISA method. HIF1A standards and samples are captured by a polyclonal HIF1A antibody on the precoated plate and detected using a biotinylated monoclonal HIF1A antibody reactive to epitopes other than the capture antibody. The biotinylated detection antibody is then bound to streptavidin-HRP, which catalyzes the conversion of TMB to a colored derivative. Color development is linear for the assay's dynamic range and directly proportional to the amount of HIF1A present in the sample.

The HIF1A protein is the 92 kDa alpha subunit of transcription factor hypoxia-inducible factor-1 (HIF-1), which is a heterodimer composed of an alpha and a beta subunit. HIF-1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation

to hypoxia. HIF-1 thus plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. Activation requires recruitment of transcriptional co-activators 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. The HIF-1 heterodimer binds to core DNA sequence 5'-[AG]CGTG-3' within the hypoxia response element (HRE) of target gene promoters.

Materials Required

- 8-well strip plates, clear, corner notched (Product No. 15031)
- Plate sealers for 96-well plates (Product No. 15036)
- Reagent reservoir, sterile, 50mL capacity, 40pk (Product No. 15075)

ELISA Reagent Kit Buffers

- D-PBS: 0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4, 0.2µm filtered (e.g., Thermo Scientific BupH Modified Dulbecco's Phosphate Buffered Saline Packs, Product No. 28374)
- Blocking Buffer: 4% BSA, 5% sucrose in D-PBS, 0.2µm filtered OR Thermo Scientific StartingBlock (PBS) Blocking Buffer, Product No. 37538 or ELISA Blocker Blocking Buffer, Product No. N502
- Reagent Diluent: 4% BSA in D-PBS (pH 7.4), 0.2µm filtered
- Wash Buffer: 0.05% Tween™-20 Detergent (e.g., 0.5% Thermo Scientific Surfact-Amps Detergent Solution, Product No. 28320) in D-PBS, pH 7.4 OR ELISA Wash Buffer (30X), Product No. N503
  Note: Mix new solution daily.

Assay Protocol

Kit components are titered to give optimal results using the HIF-1A ELISA Reagent Kit Protocol for cell culture supernatants and serum. Any change, including component concentration, volumes, incubation times or temperatures, buffer content or number of wash steps may significantly affect the ELISA results and require optimization to give the best results.

Note: Allow all reagents and buffers to equilibrate to room temperature (22-25°C) before use. Thaw one aliquot of coating and detecting antibody for each plate. Do not use a water bath.

A. Plate Preparation

1. Dilute the Coating Antibody 1:100 in D-PBS buffer by adding 110µL Coating Antibody to 10.89mL of D-PBS buffer.
2. Add 100µL of diluted Coating Antibody to each well. Cover plate with plate sealer and incubate overnight at room temperature.
3. Aspirate Coating Antibody solution and add 300µL of Blocking Buffer to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
4. Aspirate Blocking Buffer and proceed to assay or allow to dry overnight at room temperature. When sealed with desiccant, plates can be stored at 2-8°C for 6 months.

B. Assay Procedure

1. Reconstitute standard with DI water with volume stated on vial label. The concentration of the reconstituted standard is 40,000pg/mL.
2. Dilute reconstituted standard 1:2 in Reagent Diluent to prepare top Standard (20,000pg/mL). Using Reagent Diluent, prepare 1:2.5 serial dilutions of top Standard and dilute any supernatant or serum expected to read above the top standard. Add 50µL of sample or Standard to each well. Cover plate with plate sealer and incubate for 2 hours at room temperature on a plate shaker set to mild agitation.
3. Aspirate and wash three times with Wash Buffer using 300µL per well.
4. Dilute the Detection Antibody 1:100 in Reagent Diluent by adding 110µL of Detection Antibody to 10.89mL of Reagent Diluent.
5. Add 50µL of Detection Antibody to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature on a plate shaker set to mild agitation.
6. Aspirate and wash three times with Wash Buffer, using 300µL per well.
7. Dilute Streptavidin-HRP 1:400 in Reagent Diluent by adding 30µL of Streptavidin-HRP to 12mL of Reagent Diluent.

8. Add 50µL of diluted Streptavidin-HRP reagent to each well. Cover plate with plate sealer and incubate for 30 minutes at room temperature on a plate shaker set to mild agitation.
9. Aspirate and wash three times with Wash Buffer, using 300µL per well.
10. Add 100µL of Substrate Solution to each well. Cover plate with plate sealer and incubate in the dark for 30 minutes at room temperature.
11. Stop the reaction by adding 100µL of Stop Solution to each well.
12. Measure the absorbance at A450 minus A550.

C. Absorbance Measurement

Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure the absorbance at 450nm only.

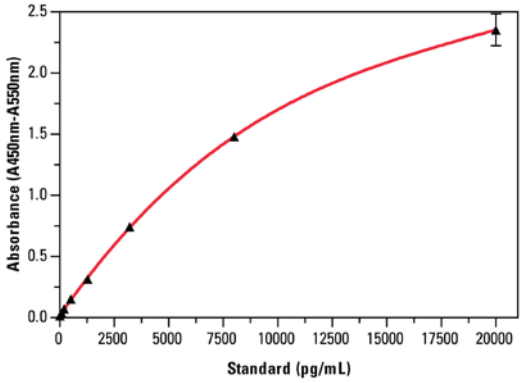
Note: When the 550nm measurement is omitted, absorbance values will be higher.

Note: Evaluate the plate within 30 minutes of stopping the reaction.

D. Calculation of Results

1. The standard curve is used to determine HIF-1A amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding HIF-1A concentration (pg/mL) on the horizontal (X) axis.
2. Calculate results using curve-fitting statistical software. Determine the HIF-1A amount in each sample by interpolating from the absorbance value (Y-axis) to HIF-1A concentration (X-axis) using the standard curve.
3. If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/mL of HIF-1A in the sample.
4. Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Standard Curve Example



Standard curve based on data obtained using the Human HIF-1A ELISA Reagent Kit Protocol.

NOTE: This standard curve is for demonstration only. A standard curve must be run with each assay.

Performance Characteristics

Specificity: This ELISA is specific for the measurement of natural and recombinant human HIF-1A. The kit shows cross-reactivity with mouse HIF-1A and is known to cross-react with rat HIF-1A.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce