Revised Protocol

Product Manual

His-Tag Protein ELISA Kit

Catalog Numbers

AKR-130 96 wells

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

A polyhistidine-tag, or His-tag, is an amino acid sequence in proteins that consists of at least five histidine (His) residues, usually inserted at the N- or C-terminus of the protein. Often used for affinity purfication of recombinant proteins in *E. coli* and other prokaryotic or eukaryotic expression systems, the 6xHis-tag motif provides a powerful purification tool while minimizing any effect on the protein's functionality and bioactivity. These attributes make it a popular tag for recombinant protein production and isolation. However, the accurate quantitation of His-tagged protein in unpurified samples remains difficult.

Cell Biolabs' His-Tag Protein ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of His-tagged protein samples (C- or N-terminal). The quantity of His-tag in a protein sample is determined by comparing its absorbance with that of a known His-tag protein standard curve (provided in the kit). The kit has detection sensitivity range of 4 µg/mL to 1 ng/mL (10 kDa His-tag protein standard), or 400 nM - 100 pM 6xHis-tag residues. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and His-tag samples. The His-Tag Protein ELISA Kit is intended for research use only, and not for diagnostic applications.

Assay Principle

The unknown His-tag samples or recombinant His-tag protein standards are first added to a polyhistidine coated plate. After a brief incubation, an anti-6xHis monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The His-tag protein content in unknown samples is determined by comparing with a standard curve that is prepared from predetermined His-tag protein standards.

Related Products

1. AKR-100: β-Galactosidase Staining Kit
2. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit

Kit Components (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate
2. Polyhistidine Conjugate (1000X) (Part No. 213001): One 20 µL vial
3. Conjugate Coating Solution (Part No. 213004): One 20 mL bottle
4. Anti-6xHis Monoclonal Antibody (Part No. 213002): One 15 µL vial
5. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial
6. Assay Diluent (Part No. 310804): One 50 mL bottle
7. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle
9. Stop Solution (Part. No. 310808): One 12 mL bottle
10. Recombinant His-Tag Protein Standard (Part No. 213003): One 20 µL vial of 4 mg/mL recombinant, C-terminal His-Tag Rhotekin RBD protein (10 kDa) in 6M GuHCl/PBS

Materials Not Supplied

1. His-Tag Protein Sample: purified or unpurified sample (cell or tissue lysate)
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Store this kit at 4ºC.

Preparation of Reagents

- Polyhistidine Coated Plate: Determine the number of wells to be used, and dilute the Polyhistidine Conjugate 1:1000 in Conjugate Coating Solution. Add 100 µL of diluted Polyhistidine Conjugate to each well of the 96-well Protein Binding Plate. Incubate for 2 hrs at 37ºC or overnight at 4ºC. Remove the Conjugate Coating Solution and wash once with dH_2O. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

Note: The Polyhistidine Coated Plate is not stable long-term. We recommend using it within 24 hrs after coating.

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-6xHis Monoclonal Antibody: Immediately before use dilute the Anti-6xHis Monoclonal Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
- Secondary Antibody, HRP Conjugate: Immediately before use dilute the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of His-Tag Protein Standards

Centrifuge the Recombinant His-Tag Protein Standard tube and mix well by titurating. Freshly prepare a dilution series of His-Tag Standard in the concentration range of 4 µg/mL – 1 ng/mL by diluting the His-tag Protein Standard stock solution in Assay Diluent (Table 1) or desired compatible lysis buffer.

Standard Tubes	Recombinant His-Tag Protein Standard (µL)	Assay Diluent or Desired Lysis Buffer (µL)	His-Tag Protein Standard Concentration	His-Tag Residue Concentration (nM)
1	2	1998	4 µg/mL	400
2	100 of Tube #1	300	1 µg/mL	100
3	100 of Tube #2	300	250 ng/mL	25
4	100 of Tube #3	300	62.5 ng/mL	6.25
5	100 of Tube #4	300	15.6 ng/mL	1.56
6	100 of Tube #5	300	3.9 ng/mL	0.39
7	100 of Tube #6	300	1 ng/mL	0.10
8	0	300	0	0

Table 1. Preparation of His-Tag Protein Standard Curve.
Note: Protein standards should be diluted in the same buffer as prepared samples (see compatibility table below).

Preparation of Samples

Samples may be prepared in assay diluent or desired lysis buffer. However, some common detergents and denaturants have been tested for compatibility in the assay (below table). Dilution of samples, and interfering substances, may be necessary for assay compatibility.

Substance	Compatible Concentration
Triton X-100	≤1%
Imidazole, pH 7.0	≤125 mM
Guanidine HCl	≤125 mM
Urea	≤500 mM
Deoxycholic Acid	≤0.5 %
SDS	≤0.05%
TBS	Compatible
PBS	Compatible
RIPA Lysis Buffer (1% Triton X-100, 1% DOC, 0.1% SDS)	≥2-fold dilution

Table 2. Substance Compatibility Table.

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use.
2. Each His-tag sample, His-tag standard, and blank should be assayed in duplicate.
3. Add 50 µL of His-tag sample or standard to the Polyhistidine Coated Plate (see Preparation of Reagents). Incubate at room temperature for 10 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-6xHis Monoclonal Antibody (see Preparation of Reagents Section) to each tested well.
5. Incubate at room temperature for 2 hours on an orbital shaker.
6. Remove Plate Cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
7. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate (see Preparation of Reagents Section) to each tested well.
8. Incubate at room temperature for 1 hour on an orbital shaker.
9. Remove Plate Cover and empty wells. Wash the strip wells 5 times according to step 6 above.
10. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 2-30 minutes on an orbital shaker.

    Important Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. 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).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical His-Tag Protein ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

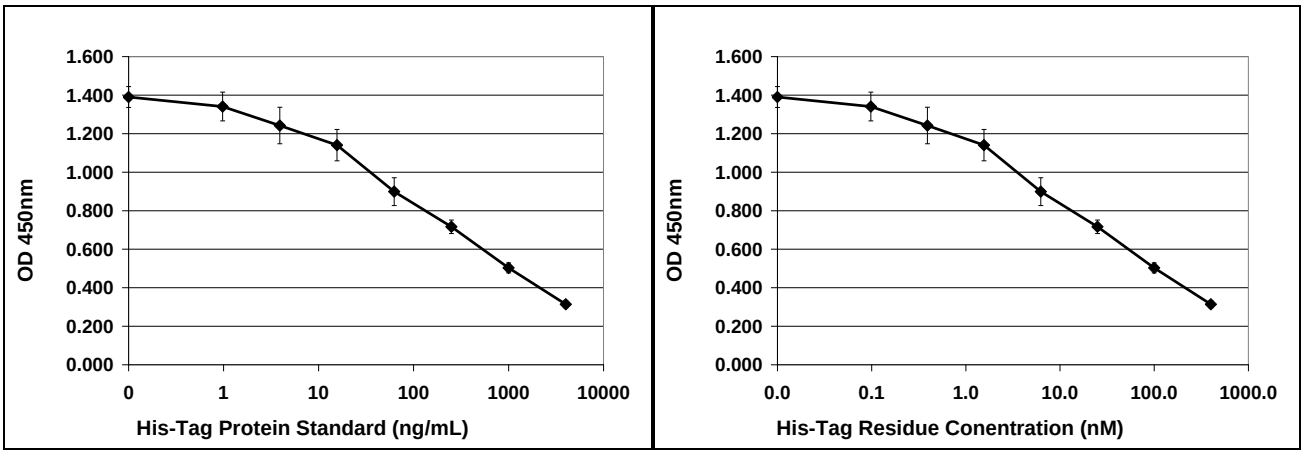

Figure 1: His-Tag Protein Standard Curve. The Recombinant His-Tag Protein Standard (Purified Recombinant, **C-terminal** His-Tag Rhotekin RBD Protein) was diluted in Assay Diluent and assayed as described in the assay instructions.

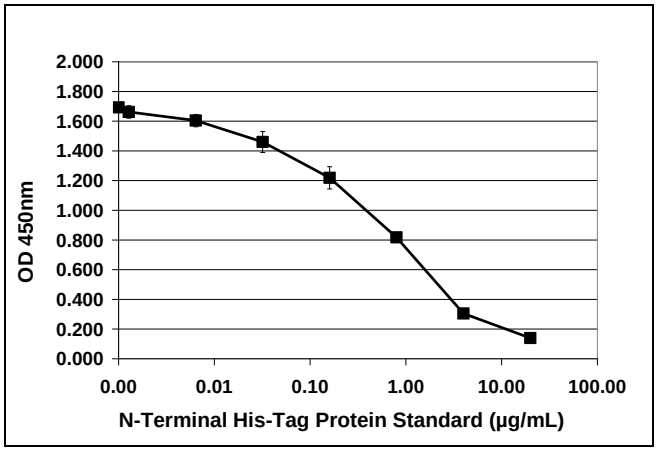

Figure 2: Quantitation of N-Terminal His-Tag Protein. Purified recombinant, **N-terminal** His-Tag Cdc42 protein was diluted in Assay Diluent and assayed as described in the assay instructions.

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Recent Product Citations

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