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Datasheet for ABIN2895503

His Tag ELISA Kit

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Overview

Quantity:	96 tests
Target:	His Tag
Reactivity:	Tag
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	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.
Sample Type:	Cell Samples, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	1 ng/mL
Characteristics:	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

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Product Details

	samples. The His-Tag Protein ELISA Kit is intended for research use only, and not for diagnostic applications.
Components:	1. 96-well Protein Binding Plate : One strip well 96-well plate 2. Polyhistidine Conjugate (1000X) : One 20 µL vial 3. Conjugate Coating Solution : One 20 mL bottle 4. Anti-6xHis Monoclonal Antibody (100X) : One 75 µL vial 5. Secondary Antibody, HRP Conjugate (1000X) : One 50 µL vial 6. Assay Diluent : One 50 mL bottle 7. 10X Wash Buffer : One 100 mL bottle 8. Substrate Solution : One 12 mL amber bottle 9. Stop Solution (Part. No. 310808): One 12 mL bottle 10. Recombinant His-Tag Protein Standard : One 20 µL vial of 4 mg/mL recombinant, C-terminal His-Tag Rhotekin RBD protein (10 kDa) in 6M GuHCl/PBS 2
Material not included:	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)

Target Details

Target:	His Tag
Alternative Name:	His-Tag Protein (His Tag Products)
Target Type:	Tag
Background:	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.

Application Details

Comment:	• Quantify proteins with His-tag at either N-terminus or C-terminus • Detect as low as 1 ng/mL of protein or 50 pM 6xHis-tag residues • Suitable for cell or tissue lysates

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Application Details

Plate:	Uncoated
Reagent Preparation:	• 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 37 °C or overnight at 4 °C. Remove the Conjugate Coating Solution and wash once with dH2O. 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 4 °C 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 1:100 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. 3 Standard Recombinant Assay Diluent or His-Tag His-Tag Protein Desired Lysis Protein His-Tag Tubes Standard Residue Standard (µL) Buffer (µL) Concentration Concentration 1 2 1998 4 µg/mL 400 nM 2 100 of Tube #1 300 1 µg/mL 100 nM 3 100 of Tube #2 300 250 ng/mL 25 nM 4 100 of Tube #3 300 62.5 ng/mL 6.25 nM 5 100 of Tube #4 300 15.6 ng/mL 1.56 nM 6 100 of Tube #5 300 3.9 ng/mL 391 pM 7 100 of Tube #6 300 1 ng/mL 98 pM 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).
Sample Preparation:	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, >2-fold dilution 0.1 % SDS) Table 2. Substance Compatibility Table.
Assay Procedure:	1. Prepare and mix all reagents thoroughly before use. 2. Each His-tag sample, His-tag standard, and blank should be assayed in duplicate. 4 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.

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Application Details

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 2-20 minutes on an orbital shaker. 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. 5

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store this kit at 4°C.

Publications

Product cited in:

Warnock, Wilson, Patten, Fleming, Maule, Dalzell: "Nematode neuropeptides as transgenic nematicides." in: **PLoS pathogens**, Vol. 13, Issue 2, pp. e1006237, (2017) (PubMed).

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Akiyama, Miyata, Komiyama, Nogami, Ozawa, Oshita, Kume, Ashizawa, Sakura, Mochizuki, Yamaguchi: "The identification of affinity peptide ligands specific to the variable region of human antibodies." in: **Biomedical research (Tokyo, Japan)**, Vol. 35, Issue 2, pp. 105-16, (2014) (PubMed).

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