ab285248 – His-tag Protein ELISA Kit

For quantitative quantitative measurement of His-Tag Protein in bacterial, insect and mammalian cell lysates, downstream analyses of purification of His-tag proteins, His-tag proteins used in protein-protein interaction studies.

For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285248

Introduction

A His-tag is an amino acid motif that contains at least six consecutive histidine (His) residues, often at the N- or C-terminus of a protein. More than 50% of recombinant proteins expressed by eukaryotic or prokaryotic expression systems are tagged with His-tag. Selective, sensitive and quantitative detection of protein levels and protein-protein binding partners expressed with His-tag is therefore a valuable tool in Life Science research. His-tag Protein ELISA Kit (ab285248, E4550) is a competitive-based ELISA that is easier, faster and more sensitive method to detect His-tag proteins expressed in both bacterial, insect and mammalian cells.

Applications

In vitro quantitative determination of His-tag proteins

Detection Range: 25.6 – 3200 ng/ml

Sensitivity: 20 ng/ml

Storage and Stability

The entire kit may be stored at -20°C for up to 12 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
His-Tag Coated Plate/ELISA Microplate	8 x 12 wells	-20°C
His-Tag Standard/Standard (MW 60.1 kDa)	2 vials	-20°C
Goat-Anti Rabbit HRP Conjugate I/HRP-conjugate stock	25 µL	-20°C
His-Tag Antibody/Antibody stock	20 µL	-20°C
TMB Substrate I/TMB Substrate	10 mL	-20°C
Stop Solution VIII/Stop Solution	10 mL	-20°C
Sample Diluent IV/Sample Diluent	20 mL	-20°C
Antibody Diluent	7 mL	-20°C
10X Wash Buffer II/Wash Buffer (10X)	50 mL	-20°C
Conjugate Buffer I/Conjugate Buffer	7.5 mL	-20°C
Microplate Sealing Film/Plate Sealers	4 units	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Cell lysis buffer (Cat. No. ab288311)
- Clean Eppendorf tubes for preparing standards or sample dilutions

Reagent Preparation

- Bring all reagents to room temperature (RT) before use.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

HRP-conjugate, TMB Substrate I/TMB Substrate, Stop Solution VIII/Stop Solution and Sample Diluent IV/Sample Diluent: Ready to be used. After use, store them at 4°C

10X Wash Buffer II/Wash Buffer: Bring bottle to RT. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer II/Wash Buffer by diluting 10 ml of 10X Wash Buffer II/Wash Buffer (10X) with 90 ml deionized water. Diluted Wash Buffer II/Wash Buffer can be stored at 4°C for 1 month.

His-Tag Antibody/Antibody Stock Spin briefly before opening the tube. Add 20 µl of His-Tag Stock/Antibody Stock into Antibody Diluent bottle (7 ml) and vortex briefly to prepare antibody solution. After use, the antibody solution can be stable at 4°C (do not freeze) for 2 months. The unused His-Tag Stock/Antibody stock should be kept at -20°C.

Goat-Anti Rabbit HRP Conjugate I/HRP-conjugate Stock: Spin briefly before opening the tube. Pipet 4 µl of Goat-Anti Rabbit HRP Conjugate I/HRP-conjugate Stock into Conjugate Buffer I/Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.

Standard Preparation

- Add 100 µl of water into a vial of His-Tag Standard/Standard to prepare standard stock. Mix 25 µl of the standard stock with 750 µl of water to prepare S5 standard (3200 ng/ml) in below. Dilute the S5 standard by 2-folds (eg. 100 µl in 100 µl of water) to prepare S4 standard. Perform 5-fold serial dilutions from S5 (e.g. 100 µl in 400 µl of water) to prepare S3 to S1 standards sequentially. S0 is water only. His-Tag Standard/Standard and diluted standards are stable at -20°C for 2 weeks. Avoid freeze and thaw cycles.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ng/mL)	0	25.6	128	640	1600	3200

Sample Preparation

- Always prepare a sample without His-tag protein expression vector (or empty vector) as control to subtract background. This is to account for proteins that may have some cross-reactivity with the antibody, e.g., those due to presence of a string of His or other backgrounds, which should be quite little. This will show as high OD readings. After obtaining the OD of the background control and samples, convert the OD to concentrations of His-Tag protein for both the samples and the background control using the standard curve. Then subtract the concentration of background control away from the concentrations of the samples.

E. Coli:

Spin 1.5 ml of E. coli cells (OD at 600 nm > 1.5) in an Eppendorf tube at 10,000 g for 2 min.
Collect the pellet and discard the supernatant.
Add 100 µl of cell lysis buffer into the sample tube, pipette to disperse the pellet and incubate at RT for 10 min.
Spin the tube and collect only the supernatant.
Dilute the supernatant 5 folds (eg. 40 µl in 160 µl of Sample Diluent IV/Sample Diluent).
Use 50 µl per well for the assay.
Δ Note: *Dilution Factor 5*

Mammalian Cells:
Take 1.5 ml of mammalian cells (10^5 – 10^6 cells/ml) in an Eppendorf tube and spin at 10,000 g for 2 min.
Transfer the supernatant to a new tube and keep the pellet in the original tube.
To detect His-tag protein in the medium, dilute the medium 2 folds (eg. 100 µl in 100 µl of Sample Diluent IV/Sample Diluent).
Use 50 µl per well for the assay.
To detect His-tag protein in cell pellet, add 100 µl of cell lysis buffer into the pellet, pipette up and down to disperse the pellet and incubate the tube at RT for 10 min.
Spin the cells and collect only the supernatant.
Dilute the supernatant 5 folds (eg. 40 µl in 160 µl of Sample Diluent IV/Sample Diluent).
Use 50 µl per well for the assay.
Δ Note: *Dilution factor: 2 for cell culture medium and 5 for cell pellet*

Assay Procedure

- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. Prepare all wells, reagents, standards and samples as described in previous sections. Put all unused wells back to -20°C. (Opened plate is stable for 1 month.)
2. Add 50 µl of standard or sample per well. Then add 50 µl of conjugate working solution and 50 µl of antibody solution to the above wells.
3. Cover the plate with plate sealer and mix well. Incubate the plate at RT for 45 min.
4. Aspirate all reagents and wash each well 4 times: add 250 µl of 1X Wash Buffer II/Wash Buffer and incubate for 30 seconds. Remove Wash Buffer II/Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times. Remove the last wash by aspiration.
5. Add 100 µl of TMB Substrate I/TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for no His-tag standard (S0). When its reading is approximately between 0.95 - 1.05 (usually between 5-20 min after adding the TMB Substrate I/TMB Substrate), add 50 µl of Stop Solution VIII/Stop Solution to each well and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm within 10 min.

Calculation

The Standard Curve is done by plotting the OD at 450 nm vs. His-tag standard concentrations. The concentration of His-tag protein in each sample can be read from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support