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His-Tag Protein ELISA Kit

rev 05/19

(Catalog # E4550-100; 100 assays, Store kit at -20°C)

I. 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. BioVision's His-Tag Protein ELISA Kit 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. This detection kit offers ready-to-use reagents, and can detect as low as 20 ng/ml of His-tag protein within 90 min.

II. Applications:

In vitro quantitative determination of His-tag proteins
Detection Range: 25.6 – 3200 ng/ml
Sensitivity: 20 ng/ml

III. Sample Type:

Bacterial, insect and mammalian cell lysates
Downstream analyses of purification of His-tag proteins
His-tag proteins used in protein-protein interaction studies

IV. Kit Contents:

Components	E4550-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4550-100-1
Standard	2 vials	Red	E4550-100-2
HRP-conjugate stock	25 µl	Blue	E4550-100-3
Antibody Stock	20 µl	Orange	E4550-100-4
TMB Substrate	10 ml	Amber	E4550-100-5
Stop Solution	10 ml	NM/Blue	E4550-100-6
Sample Diluent	20 ml	NM	E4550-100-7
Antibody Diluent	7 ml	NM/Red	E4550-100-8
Wash Buffer (10X)	50 ml	NM	E4550-100-9
Conjugate Buffer	7.5 ml	NM/Green	E4550-100-10
Plate Sealers	4	--	E4550-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Cell lysis buffer (BioVision Cat. No. 1067)
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

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

VII. Reagent and Standard Preparation:

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

- **HRP-conjugate, TMB Substrate, Stop Solution and Sample Diluent:** Ready to be used. After use, store them at 4°C.
- **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 by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. Diluted Wash Buffer can be stored at 4°C for 1 month.
- **Antibody Stock**: Spin briefly before opening the tube. Add 20 µl of 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 Antibody stock should be kept at -20°C.
- **HRP-conjugate Stock**: Spin briefly before opening the tube. Pipet 4 µl of HRP-conjugate Stock into 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**: Add 100 µl of water into a vial of 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. 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

VIII. Sample Preparation for determination of expression levels:

Notes: Always prepare a sample without His-tag protein expression vector (or empty vector) as control to subtract background.

- **E. coli**

1. 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.
2. Collect the pellet and discard the supernatant.
3. Add 100 µl of cell lysis buffer into the sample tube, pipette to disperse the pellet and incubate at RT for 10 min.
4. Spin the tube and collect only the supernatant.
5. Dilute the supernatant 5 folds (eg. 40 µl in 160 µl of Sample Diluent).
6. Use 50 µl per well for the assay.

Note: Dilution factor: 5

- **Mammalian Cells**

1. 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.
2. Transfer the supernatant to a new tube and keep the pellet in the original tube.
3. To detect His-tag protein in the medium, dilute the medium 2 folds (eg. 100 µl in 100 µl of Sample Diluent).
4. Use 50 µl per well for the assay.
5. To detect His-tag protein in cell pellet, add 100 µl of cell lysis buffer (Cat. No. 1067-100) into the pellet, pipette up and down to disperse the pellet and incubate the tube at RT for 10 min.
6. Spin the cells and collect only the supernatant.
7. Dilute the supernatant 5 folds (eg. 40 µl in 160 µl of Sample Diluent).
8. Use 50 µl per well for the assay.

Note: Dilution factor: 2 for cell culture medium and 5 for cell pellet.

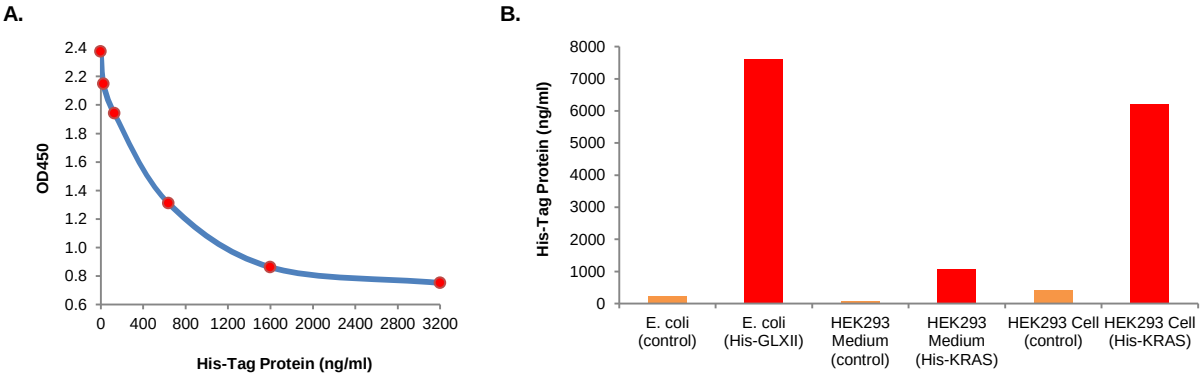
IX. His-Tag Protein ELISA Assay Protocol:

Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time an assay is performed.

1. Prepare all wells, reagents, standards and samples as described in sections VII and VIII respectively. 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 and incubate for 30 seconds. Remove 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 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), add 50 µl of Stop Solution to each well and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm within 10 min.

X. 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.



Figures. **A.** His-tag protein standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Detection of expressed His-tag protein (GLXII or KRAS) in E. coli, HEK293 medium and cells.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Folic Acid ELISA Kit (Cat. No. 4523-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)
Cell Lysis Buffer (Cat. No. 1067-100)

FOR RESEARCH USE ONLY! Not to be used on humans.