6X His tagged Protein ELISA Kit

Competitive Enzyme Immunoassay for the quantification of 6X His tagged proteins or residues in cell or tissue lysates.

Catalog number: ARG81201

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

A polyhistidine-tag is an amino acid motif in proteins that typically consists of at least six histidine (His) residues, often at the N- or C-terminus of the protein. Polyhistidine-tags are often used for affinity purification of polyhistidine-tagged recombinant proteins expressed in Escherichia coli and other prokaryotic expression systems. The 6xHis-tag motif provides a powerful purification tool while minimizing any effect on the protein's functionality and bioactivity. [provide from Wikipedia]

PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification 6X His tagged Protein or residues (C- or N-terminal tagged) in cell or tissue lysates samples. Polyhistidine is coated onto a microtiter plate before assay. Then standards or samples are added in the wells. After a brief incubation, anti-6X His tagged Protein monoclonal antibody is added. The 6X His tagged Protein antibody in the kit will be competitively bound by 6X His tagged Protein on the plate and 6X His tagged Protein in standards or samples. After washing away any unbound antibody reagent, a secondary antibody conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of 6X His tagged Protein present in samples or standards. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of 6X His tagged Protein in the sample is then determined by comparing the O.D of samples to the standard curve.

The 6X His tagged Protein in the samples or 6X His tagged Protein in the standards compete with the 6X His tagged Protein coated on the plate for antibody binding site. High 6X His tagged Protein content in a sample or high concentration of standard results in less 6X His tagged Protein-antibody binding complex on the plate, resulting in a low signal. Conversely, low 6X His tagged Protein content in a sample or low concentration of standard result in most 6X His tagged Protein antibody binding to the 6X His tagged Protein on the plate, producing a higher signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all kit components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
96-well microplate	1 strips X 96 wells	4°C
1000X Polyhistidine Conjugate	20 µl	4°C
Conjugate Coating Solution	20 ml (Ready-to-use)	4°C
6X His tagged Protein Standard (4 mg/ml)	20 µl	4°C
1000X Anti-6X His tagged Protein Antibody	15 µl	4°C
1000X HRP-Conjugated secondary antibody concentrate	20 µl	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
10X Wash Buffer	100 ml	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

Note: The standard is a recombinant, C-terminal His-tagged protein (10 kDa) in 6M GuHCl/PBS

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all kit components at 4°C. Use the kit before expiration date.
- Briefly mix and spin down the components before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Add reagents to the plate gently using a multichannel pipette. To avoid the creation of bubbles in the well, do not mix by pipetting.
- It is highly recommended that the standards, samples and controls be

assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

Note: Protein standards should be diluted in the same buffer as prepared samples.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. Example: Add 50 ml of 10X wash buffer to 450 mL of distilled water, mix well.) Storage the diluted wash buffer at 2-8°C.
- Anti-6X His tagged Protein Antibody: Dilute the antibody immediately before use, dilute the 1000X Anti-6X His tagged Protein antibody into Assay Diluent to yield 1X antibody working solution. Do not store diluted solutions.
- HRP-conjugated secondary Antibody: Dilute the antibody immediately before use, dilute the 1000X HRP-conjugated secondary Antibody into Assay Diluent to yield 1X HRP-conjugated secondary Antibody working solution. Do not store diluted solutions.
- 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 microplate. Incubate for 2 hrs at 37°C or overnight at 4°C. Remove the Conjugate Coating Solution and wash once with distilled water. 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 for long-term storage. We recommend using it within 24 hrs after coating.

- **6X His tagged Protein standard:** Centrifuge the Standard tube and mix well before opening. Freshly prepare a series dilution of standards with Assay Diluent or desired compatible lysis buffer before use. The Assay Diluent (or desired compatible lysis buffer) serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	6X His tagged Protein concentration		Dilution Buffer (µl)	Standards (µl)
	ng/mL	nM		
S1	4000	400	1998	2 (4 mg/ml stock)
S2	1000	100	300	100 (S1)
S3	250	25	300	100 (S2)
S4	62.5	6.25	300	100 (S3)
S5	15.6	1.56	300	100 (S4)
S6	3.9	0.39	300	100 (S5)
S7	0.98	0.098	300	100 (S6)
S0	0	0	300	0

Note:

1. Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.
2. Protein standards should be diluted in the same buffer as prepared samples.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. Standards and samples should be assayed in duplicates.

1. Add **50 µl** of **the 6X His tagged Protein standards and samples** into the appropriate wells of the polyhistidine coated plate. Incubate for 10 min at room temperature on a microplate shaker.
2. Add **50 µl** of **the diluted 1X Anti-6X His tagged Protein Antibody** working solution to each well, cover the plate and incubate for **2 hours at room temperature** on a microplate shaker.
3. Remove the Plate Cover and aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **the diluted 1X HRP-conjugated secondary Antibody** working solution to each well, cover the plate and incubate for **1 hour at room temperature** on a microplate shaker.
5. **Warm TMB substrate solution** before next washing step. TMB substrate solution should be equilibrated to room temperature before use.
6. Remove the Plate Cover and aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher.

Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

7. Add 100 µl of TMB substrate solution into each well. Incubate for 2-30 min at RT on a microplate shaker. Avoid exposure to light.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
8. Add 100 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately (optional: read at 620 nm as reference wave length).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

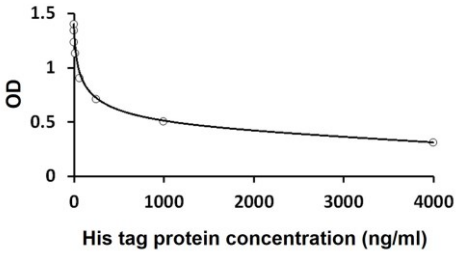
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

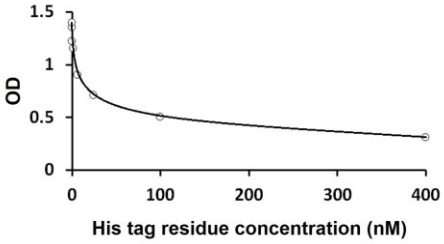
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.





QUALITY ASSURANCE

Sensitivity

1 ng/ml (Protein); 98 pM (Residue)

Assay Range

1- 4000 ng/ml (Protein); 0.098- 400 nM (Residue)

Specificity

This kit detects both at C- and N-terminal His-tagged protein in samples.