ABSbio™ His-tag Protein ELISA Kit (SE008-His)

His-tagged protein Quantification

Introduction

Epitope tags provide a convenient way to isolate interacting proteins without the need for specific antibodies to each new protein. His-tag is a short amino acid sequence consists of histidine (His) residues in recombinant proteins.

ABSbio™ His-tag Protein ELISA Kit provide a convenient, ready-to-use, high-throughput platform for rapid capture and detection of His-tagged proteins in different samples. The High Sensitivity plate is coated with the His-tagged protein and pre-blocked to provide timesaving for high-throughput users. Series dilutions of His-protein standards (28kD) and samples are added to each test well, then, anti-His tag mAb is added to the plate. His-tagged protein in solution and pre-coated His-tagged protein compete to bind the antibody. After washing steps, HRP-conjugated secondary antibody is added to each well to react with anti-His tag mAb. Finally, TMB substrates is added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when Stopping Solution is added. The yellow color intensity proportionally correlates to the concentration of the His-tagged protein standard and His-tagged protein in samples.

ABSbio's kit is a competitive ELISA that generates a reverse curve, with the lowest OD values obtained from high His concentrations, and allows you to detect and quantify His-tagged protein samples simply and reliably by comparing your unknown samples to a known recombinant standard. The kit can be used to detect N-terminal and C-terminal His-tagged proteins. This kit contains all reagents required for His tag detection, suitable for most samples, such as bacterial, yeast, mammalian cell lysates and cell culture supernatants, and the test completed within 1.5 hours. The kit can detect as little as 1 ng/mL His-tagged protein in samples.

Kit Components (96 tests)

His-tagged protein Standard:	**1 vials**	**Assay Diluent:**	**50 mL**	**TMB Solution:**	**12 mL**
Anti-His Ab (200x):	**60 µL**	**10x Wash Solution:**	**50 mL**	**Stop Solution:**	**12 mL**
HRP-conjugated Ab (200x):	**60 µL**	**Plate sealer:**	**2**	**His-tagged protein coated plate:**	**1**

Storage and Handling: Shipping on ice. Store Standard and Detection Ab at -20℃, other components at 2-8 ℃. Shelf Life: 3 months after receipt. Warm up reagents and plate to room temperature before use, keep Standard and Detection Ab on ice.

Protein Expression Screening & Quantification

- This procedure utilizes competitive ELISA method to perform protein expression screening & quantify in samples.
- **Wash Solution:** 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- **His-tagged Protein Standard:** The undiluted standard can be stored at -20°C for up to 3 months if not used immediately. Spin to bring down the material prior to open the tube. Add 10 µl of the standard to 500 µl of Assay Diluent to make the high standard concentration of 1000 ng /ml. A seven point standard curve is generated using 3-fold serial dilutions in Assay Diluent, vortex briefly for each of dilution step. Store the rest of the standard at -20°C.
- **Anti-His Detection Ab:** Immediately before use, add 30 µl of the antibody into 6 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).
- **HRP-conjugated Ab:** Immediately before use, add 55 µl of the antibody into 11 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).

Procedure Guideline

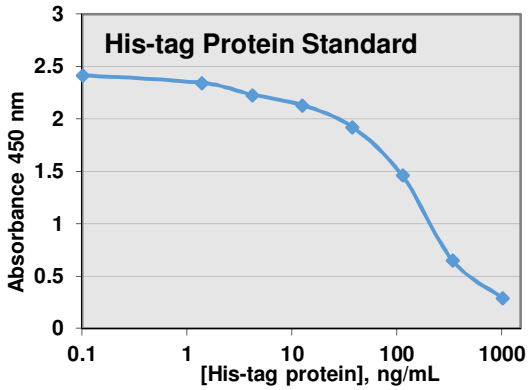
1. Prepare sample dilutions (series 10 fold dilution) in a clean 96-well plate with 1x PBS.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000ng/mL standard	1000ng/mL standard	sample1 (1:1)	sample1 (1:1)	Sample2 (1:1)	Sample2 (1:1)						
B	333.3ng/mL	333.3ng/mL	sample1 (1:10)	sample1 (1:10)	Sample2 (1:10)	Sample2 (1:10)						
C	111.1ng/mL	111.1ng/mL	sample1 (1:100)	sample1 (1:100)	Sample2 (1:100)	Sample2 (1:100)						
D	37.0ng/mL	37.0ng/mL	sample1 (1:1000)	sample1 (1:1000)	Sample2 (1:1000)	Sample2 (1:1000)						
E	12.3ng/mL	12.3ng/mL	sample1 (1:10k)	sample1 (1:10k)	Sample2 (1:10k)	Sample2 (1:10k)						
F	4.1ng/mL	4.1ng/mL	s sample1 (1:100k)	s sample1 (1:100k)	s sample2 (1:100k)	s sample2 (1:100k)						
G	1.3ng/mL	1.3ng/mL	sample1 (1:1000k)	sample1 (1:1000k)	Sample2 (1:1000k)	Sample2 (1:1000k)						
H	blank	Blank	sample1 (1:10000k)	sample1 (1:10000k)	Sample2 (1:10000k)	Sample2 (1:10000k)						

2. Set standard wells, testing sample and blank wells on the assay plate/strip as above. Transfer diluted standard 50 µl to standard wells, diluted sample 50 µl to sample wells, 50 µl Assay diluent only to blank wells. Assay should be run in duplicate. Incubate at RT for 5 mins on a micro-plate shaker at 600-700 rpm, then add 50 µl of the prepared Detection Ab to all wells.
3. Cover the plate with plate sealer and incubate the plate at room temperature for 30 mins, shaking the plate at 600-700 rpm on a micro-plate shaker.
4. Decant as much liquid as possible, fill the wells with 250 µl wash solution, shaking the plate at 600-700 rpm on a micro-plate shaker for 5 mins, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash THREE times.

5. Add 100 µl of the prepared HRP-conjugated Ab solution to all wells.
6. Cover the plate with plate sealer and incubate at room temperature for 30 mins, shaking the plate at 600-700 rpm on a micro-plate shaker.
7. Wash the plate FIVE times as described in Step#4.
8. Add 100 µl of TMB Solution to each well and incubate at room temperature for 10~20 minutes, or keep close monitoring on the developing process until desired developing blue color observed.
9. Add 100 µl of Stop Solution to each well to stop the reaction (the blue color change to yellow).
10. Read absorbance of the plate on a microplate reader at 450 nm within 15 min.
11. Average the duplicate OD readings for each standard and sample. Create a standard curve by subtracting the mean OD's for the blank. Construct standard curve (plotting the mean OD450 for each standard on the X-axis against concentration on the Y-axis, draw a best-fit curve through the points) and calculate linear regression equation, then use sample OD values and regression equation to calculate the corresponding sample concentration. It should be remembered that the sample has been diluted and its actual concentration should be justified by dilution factor (the measurement and calculation can be accomplished by software like SoftMax).
12. If molecular weight of sample differs from His-tagged standard (MW 28kD) apply the following equation to the reading concentration to obtain the actual concentration**= [MW Sample] / [MW protein standard] x Sample reading (ng/mL).**

Typical Standard Curve



Quantification of His-tagged Protein.

His-tagged protein was serially diluted and then incubated at room temperature for 30 mins with anti-His antibody in the His-tagged protein coated plate. For ELISA analysis using His-tag Protein ELISA Kit (#SE008-His), the His-tagged protein standard was incubated in the same plate. Following three wash steps. The HRP-conjugated Ab was incubated with protein-antibody complex, then the peroxidase was detected by using a soluble TMB solution for ELISA applications. The reaction was stopped using TMB stop solution. Absorbance was read on a Molecular Devices SpectraMax® at 450 nm.

Always run your own standard curves for calculation of results.

Related Products:

His-tag Protein Quick Assay Kit (#SE008-HisQ)

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.