His Tag Antibody Plate

Technical Manual No. TM0638 **Version** 01262022

Product Name	Cat.No	Size
His Tag Antibody Plate (Clear, 96-well)	L00440C	5 plates
His Tag Antibody Plate (White, 96-well)	L00440W	5 plates
His Tag Antibody Plate (Black, 96-well)	L00440B	5 plates

The product is used for rapid capture of His-tagged proteins in samples.

The operator should read technical manual carefully before using this product.

For research use only. Not for use in diagnostic procedures.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

Contents

I. Description

His tag is successive histidine (H) residues and there are mainly three forms: HHHHHH (6 x His), HHHHH (5 x His) and HHHH (4 x His). Due to its small size, less interfere in protein folding, weak immunogenicity, His tag is the most dominant tag, which is widely used in recombinant protein expression. A DNA sequence which codes for His tag, is usually constructed at N-terminus or C-terminus of variety of expression plasmids. Since His tag has high affinity for Ni^{2+} ions, it can be easily purified from bacteria, yeast and mammalian cell samples by Ni^{2+}-resin chromatography. Anti-His tag antibody is a useful tool for the analysis of His tag with different methods such as western blot, immunoprecipitation and flow cytometry.

His Tag Antibody Plate is a 96-well microtiter plate coated with THE™ His Tag Antibody. Compared with Nickel Coated Plate, it is more tolerable with several common interference reagents such as EDTA, imidazole and β-ME. The plate can bind His-tagged proteins with higher specificity and capacity. Its capacity and sensitivity varies, depending on protein size, structure and solution environment. Generally, small protein has better sensitivity than large one in a test with this product, when the plate is used to capture His-tagged proteins. Any detection reagent, which can recognize mouse IgG, **should not be** applied to the plate, such as HRP (Horseradish peroxidase) conjugated Goat anti mouse IgG and HRP-Protein A.

The product is developed for rapid capture of His-tagged proteins in biological samples. The samples include His-tagged proteins from *E.coli*, yeast and mammalian extracts and cell culture supernatant. There are several potential usages:

- Capture His-tagged proteins complexes for high throughput immuno identification assay.
- Fix His-tagged proteins on the plate, which provides a universal platform to monitor the His-tagged proteins level in different culture condition, to determine His-tagged proteins phosphorylation condition and to detect specific antibody for immunogenicity studies.
- High throughput screening of stable cell lines expressing His-tagged proteins.

GenScript provides **clear**, **white** or **black** plates respectively to satisfy different assay demands, including colorimetric, chemiluminescent or fluorescent assay.

II. Key Features

Features	Specifications
Pre-coated Antibody	THE™ Anti His Tag Antibody (Mouse monoclonal)
Specificity	N-terminal/C-terminal/internal His-tagged proteins 4 x His/5 x His/6 x His-tagged proteins
Sensitivity	1 ng/well
Capacity	150~300 ng/well
Reagents Compatibility	Tolerance of certain content of EDTA, imidazole, β-ME, etc (see detailed concentrations in the reagent compatibility table (SectionV)

III. Storage

His Tag Antibody Plate should be shipped on ice pack. The unopened plate is stable for at least 2 years when stored at 2-8 °C. The product is more stable when stored at -20 °C. The opened plate should be used within a week.

IV. Instruction for Use

- All the reagents should be equilibrated to room temperature (20-25 °C) before performing a test.
- There are general protocols for different assays. To achieve ideal test result, preliminary experiment should be carried out.
- For protein expression screening or protein quantification assay, the user should choose proper His Tag Antibody Plate and corresponding detection reagents. For example, HRP-conjugated antibody against target protein can be used for His Tag Antibody Plate (Clear, 96-well).
- Count the strips of His Tag Antibody Plate required for an assay and leave the unused strips in the foil pouch and store at 2-8 °C.

1. Immuno capture of His-tagged protein complex

- The sample volume can vary from 100~200 µl/well, depending on the amount of His-tagged proteins in sample.

Materials and Equipments Required

- **Provided in the Kit**

  His Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Test sample containing His-tagged proteins

1 x Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

PAGE Gel Sample Buffer: 1X LDS Sample Buffer (GenScript Cat#M00676) with 50mM DTT (freshly added)

Pipettor

Microtube

Procedure Guideline

1.1 Add 100 µl of test samples, negative control and positive control to different wells of His Tag Antibody Plate.

1.2 Cover the plate with plate sealer and incubate at room temperature for 1~3 hours or at 4 °C overnight.

1.3 Wash the plate four times with 260 µl of 1 x Wash Solution.

1.4 Add 30 µl of PAGE Gel Sample Buffer to each well and incubate for 10~15 minutes to elute target protein complex.

1.5 Transfer the eluted solution from the wells to the microtubes and, denature at 95°C for 5 minutes.

1.6 Store the microtubes at -20 °C or perform western blot analysis immediately.

2. Protein Expression Screening

- This procedure utilizes sandwich ELISA method to perform protein expression screening in samples.

Materials and Equipments Required

- **Provided in the Kit**

  His Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Test sample containing His-tagged proteins

  HRP-conjugated antibody against target protein

  TMB Substrate

  Stop Solution (1M HCl or 1M H_2SO_4 or 1M H_3PO_4)

  1 x Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

  Pipette

  Microplate reader capable of measuring absorbance at 450 nm

Procedure Guideline

2.1. Add 100 µl of test samples, negative control and positive control to different wells of His Tag Antibody Plate.

2.2. Cover the plate with plate sealer and incubate at room temperature for 1~3 hours or at 4 °C

overnight.

2.3. Wash the plate four times with 260 µl of 1 x Wash Solution.

2.4. Add 100 µl of prepared HRP-conjugated antibody against target protein to each well.

2.5. Cover the plate with plate sealer and incubate at room temperature for 0.5~2 hours.

2.6. Wash the plate four times with 260 µl of 1 x Wash Solution.

2.7. Add 100 µl of TMB Substrate to each well and incubate at room temperature for 10~20 minutes.

2.8. Add 50 µl of Stop Solution to each well to stop the reaction.

2.9. Read absorbance of the plate on a microplate reader at 450 nm.

3. Protein Quantification

- This procedure utilizes sandwich ELISA method to quantify His-tagged proteins in samples.
- Before test, the operator should do preliminary experiment to set up a standard curve of His-tagged proteins of interest.

Materials and Equipments Required

- **Provided in the Kit**

  His Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Test sample containing His-tagged proteins

  His-tagged protein standard

  HRP-conjugated antibody against target protein

  TMB Substrate

  Stop Solution (1M HCl or 1M H_2SO_4 or 1M H_3PO_4)

  1 x Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

  Pipettor

  Microplate reader capable of measuring absorbance at 450 nm

Procedure Guideline

3.1. Add 100 µl of prepared standards and test samples into different wells.

3.2. Cover the plate with plate sealer and incubate at room temperature for 1~3 hours or at 4 °C overnight.

3.3. Wash the plate four times with 260 µl of 1 x Wash Solution.

3.4. Add 100 µl of prepared HRP-conjugated antibody against target protein to each well.

3.5. Cover the plate with plate sealer and incubate at room temperature for 0.5~2 hours.

3.6. Wash the plate four times with 260 µl of 1 x Wash Solution.

3.7. Add 100 µl of TMB Substrate to each well and incubate at room temperature for 10~20 minutes.

3.8. Add 50 µl of Stop Solution to each well to stop the reaction.

3.9. Read absorbance of the plate on a microplate reader at 450 nm.

3.10. Generate a standard curve by plotting the absorbance on the vertical axis versus the His-tagged proteins standard concentration on the horizontal axis.

3.11. The amount of His-tagged proteins in each sample is determined by extrapolating OD values to the standard curve.

V. Reagent Compatibility

Some reagents may interfere with the test results. Check the reagents concentration in samples according to the Reagent Compatibility Tests table. Dialyse or dilute samples if needed.

Substance	Compatible Concentration
Triton X-100	≤ 2%
Imidazole	≤ 62.5 mM
Guanidine HCl	≤ 125 mM
Urea	≤ 1 M
Deoxycholic Acid	≤ 1%
SDS	≤ 0.1%
EDTA	≤ 20 mM
β-ME	≤ 160 mM
Tween-20	≤ 1%
Glycerol	≤ 1%
TBS	Compatible
PBS	Compatible
RIPA Lysis Buffer	Compatible

VI. Troubleshooting

Aassay	Problem	Probable Cause	Solution
Immuno capture of His-tagged protein complex	No signal in western blot assay	His-tagged proteins are not expressed.	Optimizing expression condition and prepare new sample
		His-tagged proteins are not captured on the plate	Check Reagent Compatibility table, to optimize reagent concentration
			Increasing incubation time with sample
			Check sample pH value. The binding performs well in neutral (pH6.8-7.4) condition.
Protein expression screening or Protein quantitation	No signal	HRP-conjugated antibody against target protein and His Tag antibody do not pair	Choose another HRP-conjugated antibody against target protein as alternative
	Weak signal	His tagged protein is low in sample	Increase incubation time with sample
			Concentrate sample
		Over diluted HRP-conjugated antibody against target protein	Increase HRP-conjugated antibody concentration
		Conjugated antibody incubation time is short	Increase antibody incubation time
		TMB Substrate incubation time is short	Increase substrate incubation time
		Incompatible reagent(s) concentration	Check and decrease concentration of reagent(s) in sample
	High Background	High concentration of HRP-conjugated antibody against target protein	Dilute HRP conjugated antibody against target protein
		Insufficient washing	Increase washing times
		Washing Solution is polluted	Use new prepared Washing Solution
		Substrate incubation time is too long	Decrease substrate incubation time

VII. Related Products

- GST Tag ELISA Detection Kit L00411
- His Tag ELISA Detection Kit L00436
- Protein A ELISA Kit L00430
- Ni-charged MagBeads L00295
- Mouse Anti-His mAb MagBeads L00275
- THE™ His Tag Antibody, mAb, Mouse A00186
- THE™ His Tag Antibody [HRP], mAb, Mouse A00612
- THE™ His Tag Antibody [Biotin], mAb, Mouse A00613
- THE™ His Tag Antibody [FITC], mAb, Mouse A01620

GenScript USA Inc.

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