

His•Tag[®] Antibody Plate

His•Tag [®] Antibody Plate	1 plate	71184-3
	5 plates	71184-4

Description

The His•Tag[®] Antibody Plate is a 96-well ELISA-compatible plate containing immobilized mouse IgG₁ His•Tag Monoclonal Antibody. The antibody is covalently attached to the surface using a method that facilitates maximal binding activity. The antibody specifically recognizes five consecutive histidines, and binds with high affinity ($K_d = 5 \times 10^{-8}$ to 1×10^{-9} M) to virtually any His•Tag fusion protein with an exposed His•Tag epitope. This plate has outstanding binding characteristics: a capacity of >100 ng His•Tag fusion protein per well, low non-specific binding, and well-to-well variation of <5%.

The His•Tag Antibody Plate can be used in a variety of assays where reliable, specific immobilization of His•Tag fusion proteins is required. It is ideal for high-throughput automation. Extracts prepared in a high-throughput 96-well format with PopCulture[™] or Insect PopCulture Reagent can be efficiently screened for expression levels using His•Tag Antibody Plates. In this protocol, two methods are described for detection of His•Tag fusion proteins using these plates: 1) Standard ELISA protocol using a primary antibody directed at the protein of interest (not including the His•Tag sequence), and 2) Detection of His•Tag/S•Tag[™] fusion protein, using the S-protein AP or HRP Conjugate.

Components

- 1 or 5 plates His•Tag Antibody Plate

Storage

His•Tag Antibody Plates are stable for 1 year at 4°C.

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Standard ELISA

In this assay, the His•Tag[®] fusion protein is captured on the His•Tag Antibody Plate and detected using another antibody specific to the target protein. The His•Tag Monoclonal antibody is a mouse IgG₁ and therefore anti-mouse secondary antibodies should not be used. This protocol is provided as a guideline. Optimal conditions should be determined empirically.

1. Prepare dilutions of the cell lysate containing the His•Tag fusion protein in 1X TBS (10 mM Tris-HCl pH 7.5, 150 mM NaCl) containing 3% BSA.
 - The recommended concentration of target protein is 0.1–1 µg/ml. As little as 1–10 ng/ml may be used, depending on the sensitivity of the antibody directed to the target protein.
 - If the concentration is unknown, prepare serial dilutions to determine the optimal concentration.
2. Add 100 µl cell lysate containing the His•Tag fusion protein per well. Incubate 1–3 h at room temperature or overnight at 4°C. Assays, including controls, should be performed in duplicate or triplicate.
 - A negative control uses cell lysate lacking target protein. Include all other reagents.
 - A reagent blank uses no cell lysate (substitute with lysate diluent). Include all other reagents.
3. Wash the wells 4 times with 200 µl 1X TBST (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1 % v/v Tween[®]-20). For each wash, incubate the wells with buffer for up to 1 min, and then completely remove the buffer.
4. Prepare dilutions of the primary antibody or primary antibody conjugate (AP or HRP) directed against the target protein (not the His•Tag sequence) with 1X TBS containing 3% BSA. If the primary antibody is directly conjugated to AP or HRP, proceed to step 10.
 - The optimal antibody concentration is usually between 0.1 and 1 µg/ml, but should be determined empirically.
5. Add 100 µl diluted antibody. Incubate 1–2 h at room temperature, or 4°C overnight.
6. Wash the wells 4 times with 200 µl 1X TBST. For each wash, incubate the wells with buffer for up to 1 min and then completely remove the buffer.
7. Prepare dilutions of AP- or HRP-conjugated secondary antibody (if required) per the manufacturer's recommendations. Do not use anti-mouse secondary antibodies because they may bind to the His•Tag Monoclonal Antibody instead of the primary antibody.
 - Use Novagen's Goat Anti-Rabbit IgG AP Conjugate (Fc specific, Cat. No. 69265-3), as the secondary detection antibody when using a Rabbit primary antibody.
8. Add 100 µl secondary antibody solution per well. Incubate for 1 h at room temperature.
9. Wash the wells 3 times with 200 µl TBST.
10. Prepare fresh substrate buffer:
 - Use 10X *p*-Nitrophenyl phosphate substrate buffer (Calbiochem, Cat. No. 487664) and *p*-Nitrophenyl Phosphate substrate (Calbiochem, Cat. No. 487663) for AP detection.
 - Use TMB (3,3',5,5' Tetramethylbenzidine) substrate for HRP detection (Calbiochem, Cat. No. 613544). TMB incubation should be done in the dark.
11. Add 100 µl substrate solution per well and incubate 15–60 min. A time course of color development may be required to determine optimal development time.
12. Stop the reaction by adding 100 µl appropriate stop solution:
 - Use 1N NaOH for *p*-Nitrophenyl phosphate.
 - Use 500 mM H₂SO₄ or 250 mM HCl for TMB.
13. Read the absorbance immediately. The signal can increase over time after the addition of stop solution. Read at 405 nm for *p*-Nitrophenyl phosphate and 450 nm for TMB.

Detection with S-protein

This procedure can be used when the target protein contains both His•Tag[®] and S•Tag[™] fusion sequences. The method is based on detection with the S-protein AP Conjugate (Cat. No. 69598-3) or HRP Conjugate (Cat. No. 69047-3). Fusion proteins bearing His•Tag/S•Tag sequences can be produced using many Novagen expression vectors.

1. Prepare dilutions of the cell lysate containing the His•Tag fusion protein in 1X TBS (10 mM Tris-HCl pH 7.5, 150 mM NaCl) containing 3% BSA.
 - The recommended concentration of target protein is 0.1–1 µg/ml. As little as 1–10 ng/ml may be used, depending on the sensitivity of the antibody directed to the target protein.
 - If the concentration is unknown, prepare serial dilutions to determine the optimal concentration.
2. Add 100 µl cell lysate containing the His•Tag/S•Tag fusion protein per well. Incubate 1 to 3 h at room temperature, or overnight at 4°C. Assays, including controls, should be performed in duplicate or triplicate.
 - A negative control uses a cell lysate lacking target protein. Include all other reagents.
 - A reagent blank uses no cell lysate (substitute with lysate diluent). Include all other reagents.
3. Wash the wells 4 times with 200 µl 1X TBS. For each wash, incubate the wells with buffer for up to 1 min and then completely remove the buffer.
4. Dilute S-protein AP (Cat. No. 69598-3) or HRP (Cat. No. 69047-3) Conjugate 1:5,000 with 3% BSA in 1X TBS.
5. Add 100 µl S-protein Conjugate solution. Incubate 1 h at room temperature.
6. Wash the wells 4 times with 200 µl 1X TBS. For each wash, incubate the wells with buffer for up to 1 min and then completely remove the buffer.
7. Prepare fresh substrate buffer:
 - Use 10X *p*-Nitrophenyl phosphate substrate buffer (Calbiochem, Cat. No. 487664) and *p*-Nitrophenyl Phosphate substrate (Calbiochem, Cat. No. 487663) for AP detection.
 - Use TMB (3,3',5,5' Tetramethylbenzidine) substrate for HRP detection (Calbiochem, Cat. No. 613544). TMB incubation should be done in the dark.
8. Add 100 µl of substrate solution and incubate 15–60 min. A time course of color development may be required to determine optimal development time.
9. Stop the reaction by adding 100 µl appropriate stop solution:
 - Use 1N NaOH for *p*-Nitrophenyl phosphate.
 - Use 500 mM H₂SO₄ or 250 mM HCl for TMB.
10. Read the absorbance immediately. The signal can increase over time after the addition of stop solution. Read at 405 nm for *p*-Nitrophenyl phosphate and 450 nm for TMB.