

DATASHEET

Version: 2016-08-17

THE™ His Tag Antibody, mAb, Mouse**Cat. No.:** A00186-100**Size:** 100 µg**Synonyms:** THE™ Anti-His mAb;**Description:**

Monoclonal antibodies specific to six histidine tags can greatly improve the effectiveness of several different kinds of immunoassays, helping researchers identify, detect, and purify polyhistidine fusion proteins in bacteria, insect cells, and mammalian cells. However, since 6XHis-tag is poorly immunogenic, it needs to be conjugated to KLH or some other carrier as an immunogen. After hundreds of selection cycles, researchers at GenScript successfully isolated an antibody against His-tag.

THE™ His Tag Antibody, mAb, Mouse (subtype IgG1) has very high affinity. Tests performed at GenScript show that the antibody can also recognize 4xHis- and 5xHis-tags. This means that even if the 6xHis-tag is only partially exposed, it will still be recognized and bound by this antibody.

THE™ His Tag mAb is produced from mice ascites and purified by protein A affinity column. This antibody recognizes native as well as denatured forms of synthetic polyhistidine and polyhistidine-tagged fusion proteins. The product reacts with fusion proteins expressed in bacteria, insect cells, and mammalian cells. **THE™ His Tag mAb** recognizes His tags placed at N-terminal, C-terminal, and internal regions of fusion proteins.

THE™ His Tag mAb can be used in Western blot analyses, Dot blot analyses, ELISA, immunofluorescent staining, and flow cytometry of cultured cells.

Immunogen: A synthetic peptide HHHHHH coupled to KLH**Host:** Mouse**Conjugation:** Unconjugated**Fusion Partner:**

Spleen cells were fused with SP2/0-Ag14 mouse myeloma cells

Example**Formulation:**

0.5 mg/ml, lyophilized with PBS, pH 7.4, containing 0.02% sodium azide

Clone: 6G2A9**Ig Subclass:** IgG1, k**Specificity: THE™ His Tag Antibody, mAb, Mouse**

recognizes C-terminal, N-terminal, and internal His tagged fusion proteins.

Purification: Protein A affinity column**Applications:**

Working concentrations for specific applications should be determined by the investigator. The appropriate concentrations may be affected by secondary antibody affinity, antigen concentration, the sensitivity of the method of detection, temperature, the length of the incubations, and other factors. The suitability of this antibody for applications other than those listed below has not been determined. The following concentration ranges are recommended starting points for this product.

ELISA: 0.05-0.2 µg/ml**Western Blot:** 0.1-0.2 µg/ml**Immunoprecipitation (IP):** 1 µg/ml**Immunofluorescent staining:** 1 µg/ml**Flow cytometry (FACS):** 1 µg/ml**TR-FRET assays:** user-optimized**Other applications:** user-optimized**Reconstitution:**

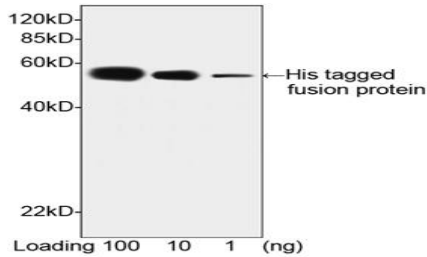
Reconstitute the lyophilized antibody with deionized water (or equivalent) to a final concentration of 0.5 mg/ml.

Storage:

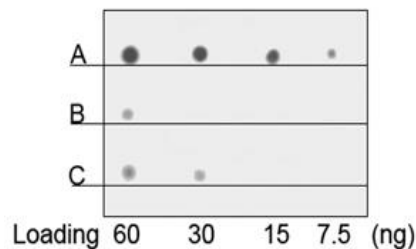
The antibody is stable in lyophilized form if stored at -20°C or below. The reconstituted antibody can be stored for 2-3 weeks at 2-8°C. For long term storage, aliquot and store at -20°C or below. Avoid repeated freezing and thawing cycles.



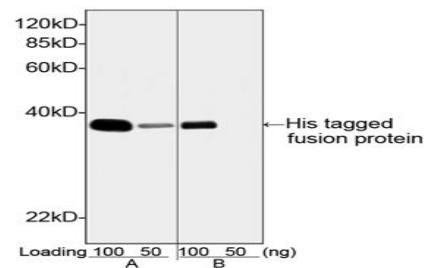
Western blot analysis of His-tagged fusion proteins using THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/ml)
Lane 1: N-terminal His-tagged fusion protein
Lane 2: C-terminal His-tagged fusion protein
The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody (GenScript, A00160, 1:10,000) and LumiSensor™ HRP Substrate Kit (GenScript, L00221).



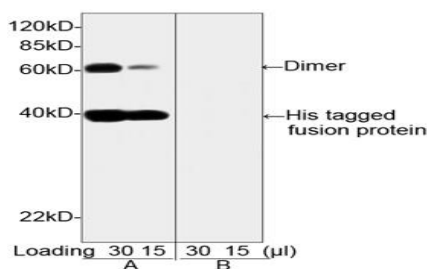
Western blot analysis of Multiple Tag Cell Lysate (GenScript, M0100) using THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/ml)
The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody (GenScript, A00160, 1:10,000) and LumiSensor™ HRP Substrate Kit (GenScript, L00221).
Predicted Size: 52 kD
Observed Size: 52 kD



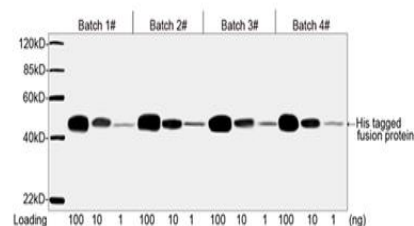
Comparison of THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, 1 µg/ml) with Mouse Anti-His mAbs (B: Competitor Q#1, 1 µg/ml; C: Competitor Q#2, 1 µg/ml) by Dot blot.
The assay was performed with His-tagged fusion protein.
The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody (GenScript, A00160, 1:10,000) and LumiSensor™ HRP Substrate Kit (GenScript, L00221).



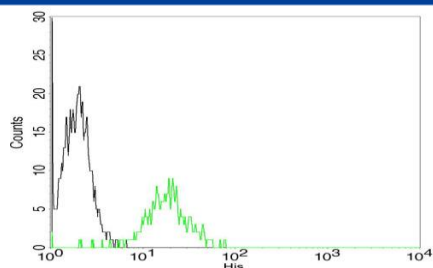
Comparison of THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, 0.1 µg/ml) with Mouse Anti-His mAb (B: Competitor A, 0.1 µg/ml) by Western blot.
The assay was performed with His-tagged fusion protein.
The signal was developed with One-Step Western™ Basic Kit (GenScript, L00205).



Comparison of THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, 1 µg/ml) with Mouse Anti-His mAb (B: Competitor B, 1 µg/ml) by Western blot.
The assay was performed with cell lysates containing His-tagged fusion protein.
The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody (GenScript, A00160, 1:10,000) and LumiSensor™ HRP Substrate Kit (GenScript, L00221).

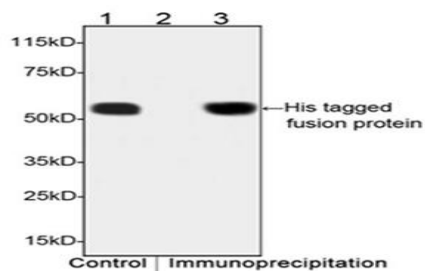


Consistency analysis of Batch 1#, 2#, 3# and 4# of THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/ml) by Western blot, showing that signal remains consistent from Lot to Lot.
The assay was performed with His-tagged fusion protein.
The signal was developed with IRDye™ 800 Conjugated Goat Anti-Mouse IgG.



Flow cytometric analysis of non-transfected or His fusion gene transfected CHO cells using **THE™ His Tag Antibody, mAb, Mouse (GenScript, A00186)** (black and green, respectively).

The signal was developed with FITC conjugated Goat Anti-Mouse IgG.



Western blot analysis of immunoprecipitates from cell lysates containing His fusion protein using THE™ His Antibody, mAb, Mouse (GenScript, A00186).

1. Positive control containing His fusion protein
2. Negative control – IP with isotype control antibody (A01007)
3. Immunoprecipitation with THE™ His Tag Antibody, mAb, Mouse (A00186)