

THE™ His Tag Antibody, mAb, Mouse

Cat. No.: A00186

Overview

Specificity	THE™ His Tag Antibody, mAb, Mouse recognizes His tags localized at the C-terminal, N-terminal, and internal region of fusion proteins.
Host Species	Mouse
Immunogen	A synthetic peptide HHHHHH coupled to KLH
Conjugate	Unconjugated

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.

Application	Recommended Usage
ELISA	0.05-0.2 µg/ml
Western Blot	0.1-0.2 µg/ml
Immunoprecipitation (IP)	1 µg/ml
Immunocytochemistry/Immunofluorescence (ICC/IF)	1 µg/ml
Flow Cytometry	1 µg/ml
Time-resolved fluorescence energy transfer (TR-FRET)	User-optimized

Properties

Form	Lyophilized
Storage Buffer	Lyophilized with PBS, pH 7.4, containing 0.02% sodium azide.

Reconstitution	Reconstitute the lyophilized powder with sterile water (or equivalent) to a final concentration of 0.5 mg/mL.
Storage Instructions	The lyophilized product remains stable up to 1 year at -20°C from date of receipt. 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.
Purification	Purified by Protein A affinity chromatography.
Isotype	Mouse IgG1, κ
Clonality	Monoclonal
Clone ID	6G2A9
Note	GenScript can offer this product according to your requirement, including product size, buffer components, etc.

Examples

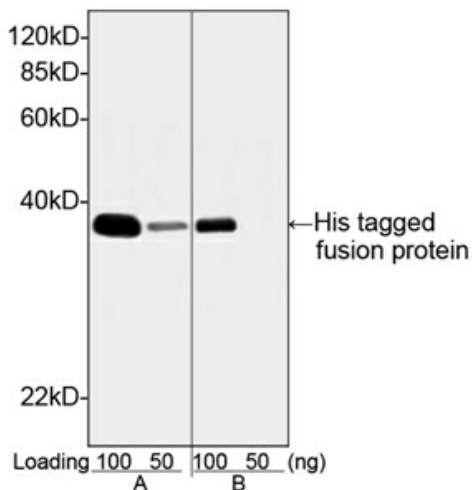


Figure 1. Comparative His-tag detection by Western blot was performed using THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, at 0.1 μ g/mL concentration) and Mouse Anti-His mAb (B: Competitor A, at 0.1 μ g/mL concentration). Both antibodies were used to probe the same sample containing overexpressed His-tagged fusion protein.

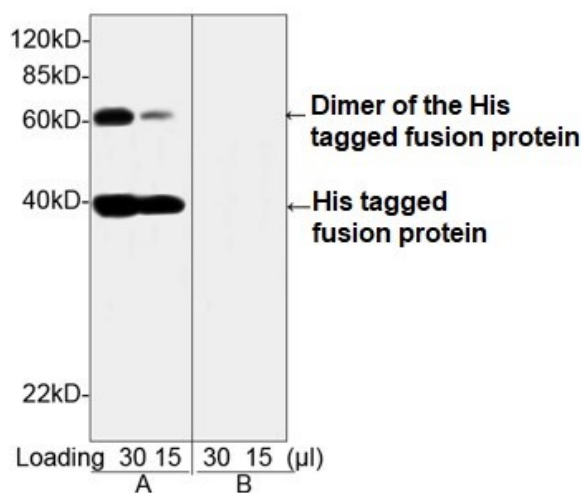


Figure 2. Comparative His-tag detection by Western blot was performed using THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, at 1 μ g/mL concentration) and Mouse Anti-His mAb (B: Competitor B, at 1 μ g/mL concentration). Both antibodies were used to probe the same cell lysates containing His-tagged fusion protein. The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody. Researchers can also use MonoRab™ Anti-Mouse IgG (H&L) (76F10), mAb, Rabbit (GenScript, V90301) as a secondary antibody.

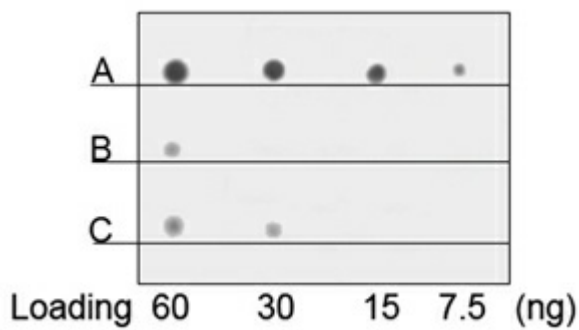


Figure 3. Comparative His-tag detection by Dot blot was performed using THE™ His Antibody, mAb, Mouse (A: GenScript, A00186, at 1 µg/mL concentration) and two Mouse Anti-His mAbs (B: Competitor Q#1, at 1 µg/mL concentration; C: Competitor Q#2, at 1 µg/mL concentration).

All three antibodies were used to probe the same samples containing His-tagged fusion protein.

The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody.

Researchers can also use MonoRab™ Anti-Mouse IgG (H&L) (76F10), mAb, Rabbit (GenScript, V90301) as a secondary antibody.

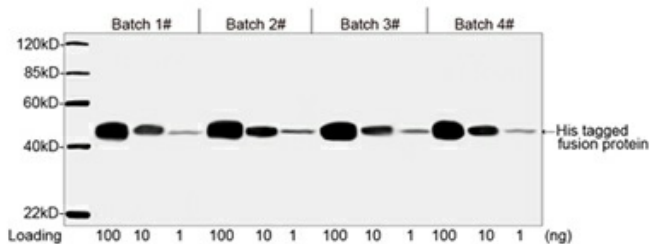


Figure 4. Lot-to-lot consistency of antibody performance was analyzed for 4 batches (Batched 1#, 2#, 3# and 4#) of THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/mL) by Western blot.

The results show that the antibody-generated signal remains consistent from Lot-to-Lot.

Antibodies from all four lots were used to probe samples of the same His-tagged fusion protein.

The signal was developed with IRDye™ 800 Conjugated Goat Anti-Mouse IgG.

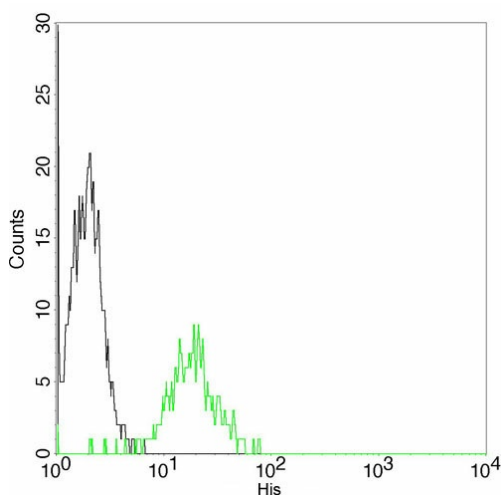


Figure 5. Detection of His-tag in CHO cells transfected with His-tagged protein (Green), and non-transfected CHO cells (Black) by flow cytometry using THE™ His Tag Antibody, mAb, Mouse (GenScript, A00186).

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

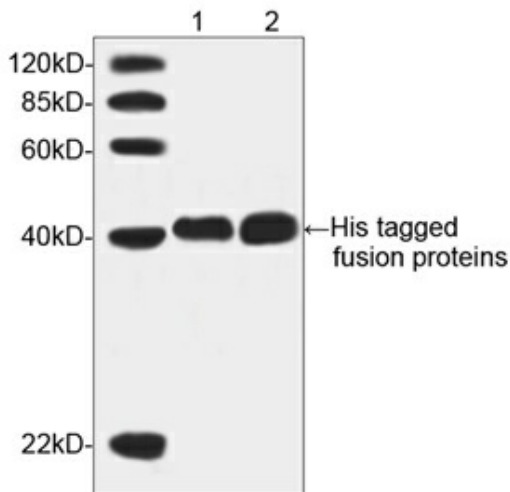


Figure 6. Detection of N- or C-terminal His tags in recombinant fusion proteins were analyzed by Western blot using THE™ His Antibody, mAb, Mouse (GenScript, A00186, at 1 µg/mL concentration).

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.

Researchers can also use MonoRab™ Anti-Mouse IgG (H&L) (76F10), mAb, Rabbit (GenScript, V90301) as a secondary antibody.

The results show that THE™ His Antibody, mAb, Mouse (GenScript, A00186) can recognize N-terminal and C-terminal His-tagged proteins.

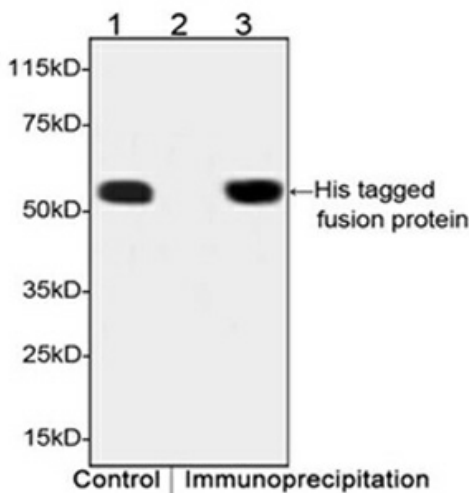


Figure 7. Immunoprecipitates from cell lysates containing His-tagged fusion protein were analyzed by Western blot using THE™ His Antibody, mAb, Mouse (GenScript, A00186).

Lane 1. Positive control containing His-tagged fusion protein

Lane 2. Negative control – IP with isotype control antibody (A01007)

Lane 3. Immunoprecipitation with THE™ His Tag Antibody, mAb, Mouse (A00186)

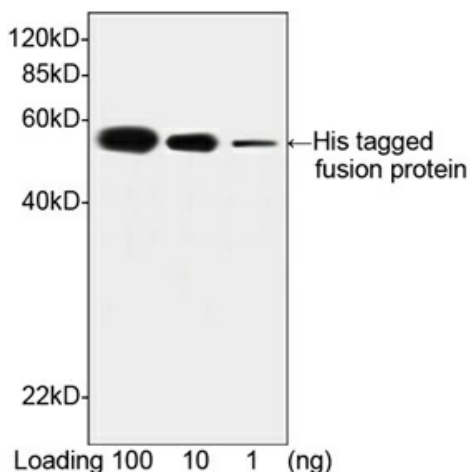


Figure 8. His-tag was detected in Multiple Tag Cell Lysate (GenScript, M0100) by Western blot using THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/mL).

The signal was developed with Goat Anti-Mouse IgG (H&L) [HRP] Polyclonal Antibody.

Researchers can also use MonoRab™ Anti-Mouse IgG (H&L) (76F10), mAb, Rabbit (GenScript, V90301) as a secondary antibody.

His-tagged fusion protein:

Predicted MW: 52 kDa

Observed MW: 52 kDa

Background

Target Background : Monoclonal antibodies specific to six histidine tag 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 the His-tag. THE™ His Tag Antibody, mAb, Mouse (subtype IgG1) has very high affinity towards the His-tag. 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. 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.

Synonyms : THE™ Anti-His mAb

For laboratory research use only. Direct human use, including taking orally and injection and clinical use are forbidden.