

THE™ His Tag Antibody, mAb, Mouse

Technical Manual No. TM0243

Cat. No. A00186

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I. DESCRIPTION

Epitope tags are widely used for detection of recombinant proteins by immunoblotting, immunoprecipitation and immunostaining techniques. Among more than a dozen of different epitope tags, His tag is one of the most popular tags to be currently utilized in protein-related research and manufacturing enterprises.

His tag is an amino acid motif in proteins that consists of at least five histidine (His) residues, often at the N- or C-terminus of the protein. It is also known as hexa histidine tag, 6xHis tag, and by the trademarked name His tag. The His tagged fusion proteins allow for efficient coupling to Ni²⁺ affinity resins and purification by single step chromatography. His tag monoclonal antibody can be used in variety of immunoassays, helping researchers to identify, detecting, and purify His tagged fusion proteins in different expression systems, such as bacteria, yeast, and mammalian cells.

GenScript **THE™ His Tag Antibody, mAb, Mouse** (Cat.No. A00186) is produced from the hybridoma that results from fusion of Sp2/0 myeloma and lymphocytes obtained from mouse immunized with synthetic peptide HHHHHH coupled to KLH. This antibody is produced from mice ascites and purified by Protein A affinity column. With superior sensitivity, THE™ His Tag Antibody, mAb, Mouse can recognize 6xHis tag as well as 4xHis and 5xHis tag. It binds His tags placed at N-terminal, C-terminal, and internal positions of His tagged fusion proteins. THE™ His Tag Antibody, mAb, Mouse can be applied to various immunoassays, including Western blot, Dot blot, ELISA, Immunofluorescent staining, Flow Cytometry and Immunoprecipitation.

II. KEY FEATURES

- **Superior Sensitivity:** It is the most sensitive product on the market.
- **High Specificity:** It detects His tagged fusion protein without non-specific binding.
- **Widespread Diversity:** It detects fusion proteins containing His tag placed at N-terminal, C-terminal, or internal regions. It also recognizes fusion proteins with different lengths of His tag (6xHis-, 5xHis-, and 4xHis-tags).
- **Long-term Stability:** It remains stable in lyophilized form for at least two years.
- **Excellent lot-to-lot Consistency:** It has been proven consistent from Lot to Lot.

III. STORAGE

THE™ His Tag Antibody, mAb, Mouse should be stored, lyophilized until use. It is stable in lyophilized form for at least two years if stored at -20°C. The reconstituted antibody can be stored for up to three months at 2-8°C or for up to two years at -20°C or below. Avoid repeated freeze and thaw cycles. Reconstitute the lyophilized THE™ His mAb (100 µg) with 200 µl of deionized water to a final concentration of 0.5 mg/ml.

IV. APPLICATIONS

THE™ His Tag Antibody, mAb, Mouse can be used in the following applications:

- **ELISA**
- **Western Blot and Dot Blot**
- **Immunoprecipitation (IP)**
- **Immunofluorescent Staining**
- **FAC Assay/Flow Cytometry**

V. EXAMPLES – ELISA

It has been confirmed THE™ His Tag Antibody, mAb, Mouse **can** reliably and specifically recognize different His-tagged peptides by ELISA. Typically, 5 µg/ml of His peptides are precoated on plates and incubated with antibody solution, followed by detection with HRP-conjugated anti-mouse IgG.

Antigen	Peptide sequence	Reactivity
4xHis	HHHH	+
5xHis	HHHHH	+++
6xHis	HHHHHH	+++

VI. EXAMPLES - WESTERN BLOT

General Protocol:

1. Transferring or spotting proteins to membrane using the following standard procedures.
2. Block the membrane with 5% nonfat dried milk in PBST for one hour at room temperature. Wash the membrane with PBST twice for five minutes each time.
3. Dilute THE™ His mAb (0.5 mg/ml) with 1% of BSA in PBST. About 0.1-0.2 µg/ml concentration is recommended. Incubate the membrane from Step 2 in the diluted antibody solution on a shaker for one hour at room temperature.
4. Rinse the membrane once with PBST Solution. Then wash the membrane on a shaker twice for five minutes each time with PBST Solution.
5. Dilute Goat anti mouse IgG conjugated with HRP (Genscript, Cat. No. A00160, 1:1000 dilution) with 5% nonfat dried milk in PBST. Incubate the membrane from Step 4 in the diluted secondary antibody solution on a shaker for one hour at room temperature.
6. Rinse the membrane once with PBST Solution. Then wash the membrane on a shaker three times for ten minutes each time with PBST Solution.
7. Then develop the blot with LumiSensor™ Chemiluminescent HRP Substrate Kit (Genscript, L00221).
8. Expose to a sheet of film for 30 seconds and develop the film. Repeat this step with different exposure times to get the best results.

THE™ His Tag Antibody, mAb, Mouse shows its superior specificity when binding to 6xHis-tagged proteins. It also recognizes proteins with 4xHis or 5xHis tags.

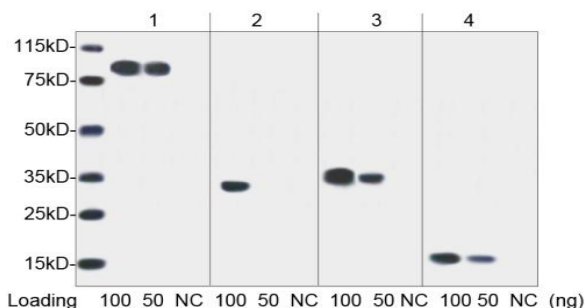


Figure 1. Western blot analysis of 6xHis tagged proteins

	Protein	Tag	Sequence
1	Pfu	C-terminalNIKKSGSSHHHHHH
2	T4 kinase	C-terminalASGNFGSSHHHHHH
3	Cart	N-terminal	MSGSHHHHHHSSGMS.....
4	Trx	InternalGSGSGMHHHHHHSSGLVP.....

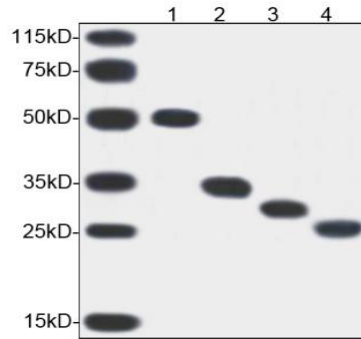


Figure 2. Western blot analysis of 4xHis and 5xHis tagged fusion proteins

	Protein	Tag	Sequence
1	GSTHHHHHGST	InternalGHTGHRSHHHHHMSPILG.....
2	HHHHHGST	N-terminal	HHHHHSSGMSPILGY.....
3	GSTHHHHH	C-terminalGHTGHRSGTHHHHH
4	GSTHHHH	C-terminalGHTGHRSGTHHHH

Superior sensitivity and specificity of THE™ His Tag Antibody, mAb, Mouse (A00186) compared from GenScript and an anonymous competitor

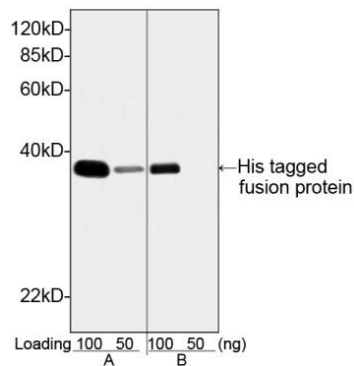


Figure 3. Western blot analysis of HHHHHH-Cart fusion protein (6xHis at N-terminal position)

A: THE™ His Antibody, mAb, Mouse (GenScript, A00186, 0.1 µg/ml)

B: Mouse Anti-His mAb (Competitor A, 0.1 µg/ml)

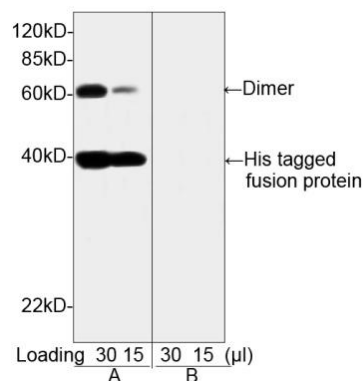


Figure 4. Western blot analysis of GST-HHHH fusion protein (4xHis at C-terminal position)

A: THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/ml)

B: Mouse Anti-His mAb (Competitor B, 1 µg/ml)

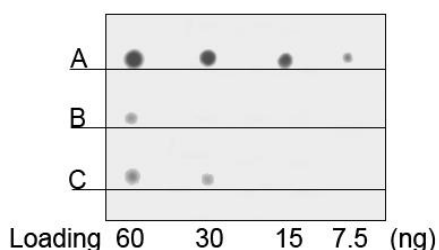


Figure 5. Dot blot analysis of HHHHH-GST fusion protein (5xHis at N-terminal)

A : THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 µg/ml)

B: Mouse Anti-His mAbs (Competitor Q#1, 1 µg/ml)

C: Mouse Anti-His mAbs (Competitor Q#2, 1 µg/ml)

The assay was performed with cell lysates containing His tagged fusion protein (5xHis).

VII. EXAMPLES - IMMUNOPRECIPITATION (IP)

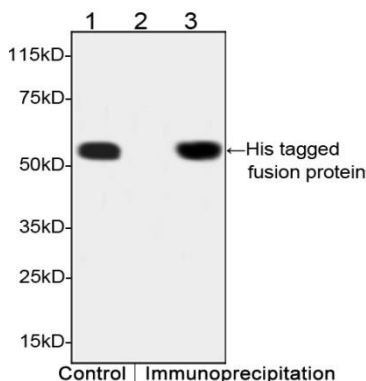


Figure 6. Western blot analysis of immunoprecipitates from cell lysates containing His fusion protein using THE™ His Tag Antibody, mAb, Mouse (Cat.No.A00186).

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)

The Immunoprecipitation was performed with ONE-HOUR IP-Western Kit (Mouse) (GenScript, Cat. No. L00232).

VIII. EXAMPLES - IMMUNOFLUORESCENT STAINING

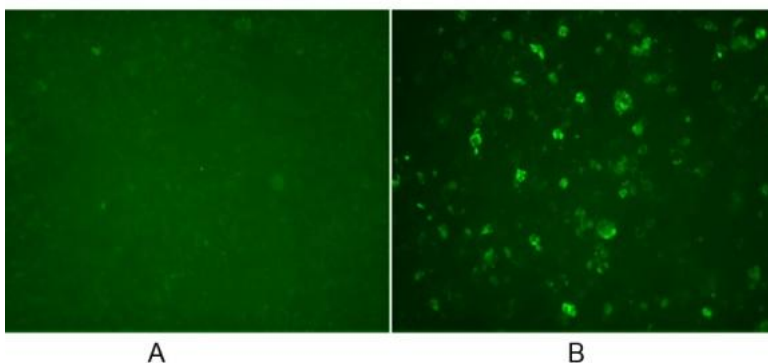


Figure 7. Immunocytochemistry/Immunofluorescence analysis of Non transfected HEK293 cells (A) and HEK293 cells transfected with His tagged fusion gene (B) using THE™ His Tag Antibody, mAb, Mouse (Cat.No.A00186)

IX. EXAMPLES - FACS ASSAY/FLOW CYTOMETRY

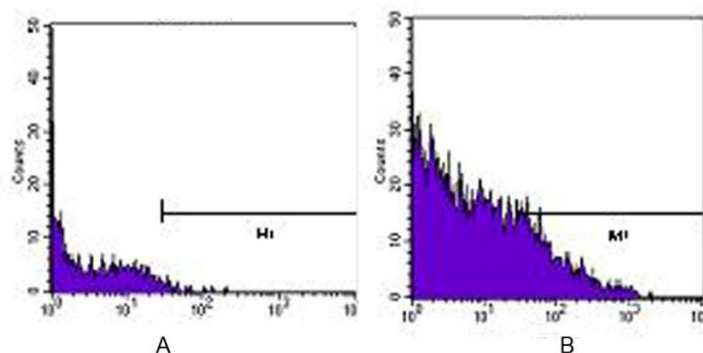


Figure 8. Flow cytometric analysis of Non-transfected HEK293 cells (A) and HEK293 cells transfected with His tagged fusion gene (B) using THE™ His Tag Antibody, mAb, Mouse

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

X. CONJUGATED ANTIBODY

HRP-, Biotin-, FITC- and magnetic beads -conjugated THE™ His Antibody, mAb, Mouse

The advantage of using a secondary antibody is that it amplifies the signal. However, if the sensitivity of the system is sufficient, working with only a primary antibody conjugated to HRP or a fluorescent dye may be preferable, since this reduces assay time by eliminating the use of secondary reagents. It also reduces the risk of non-specific background noise caused by cross-reactive secondary antibodies. If THE™ His Tag Antibody [Biotin], mAb, Mouse is to be used, Streptavidin-HRP (GenScript, Cat. No. M00091) is recommended. The optimal dilution should be determined by the user.

Magnetic bead conjugated antibody is available for you to achieve rapid and efficient biomagnetic separation and ensure reliable and reproducible results.

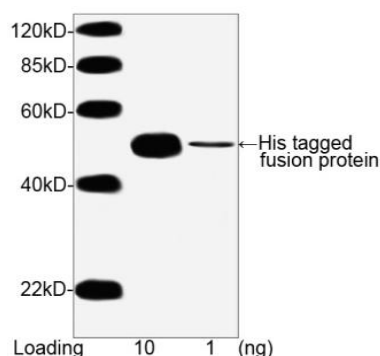


Figure 9. Western blot analysis of His tagged fusion protein (Genscript, M0100) using THE™ His Tag Antibody [Biotin], mAb, mouse (GenScript, A00613, 1 µg/ml)

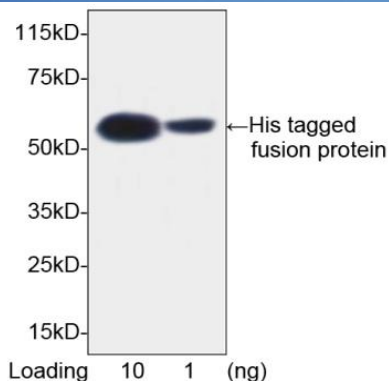


Figure 10. Western blot analysis of His tagged fusion protein (Genscript, M0100) using THE™ His Tag Antibody [HRP], mAb, Mouse (GenScript, A00612, 1 µg/ml)

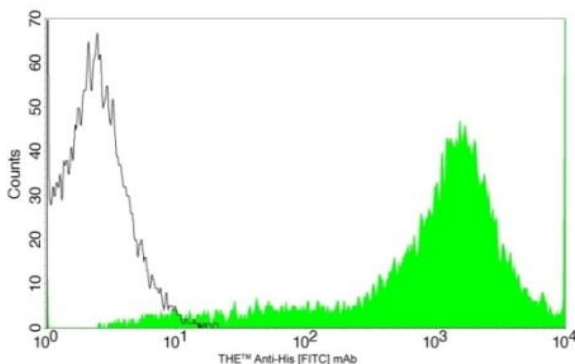


Figure 11. Flow cytometric analysis of HEK293 cells transfected with His-tagged gene using THE™ His Tag Antibody [FITC], mAb, Mouse (GenScript, A01620; shaded histogram) or with a negative control antibody (open histogram).

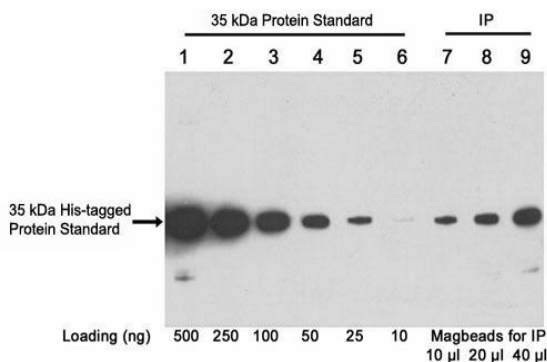


Figure 12. 10 µl, 20 µl and 40 µl of THE™ His Tag Antibody MagBeads were used to purify (or immunoprecipitate) a 35 kDa His-tagged protein from *E. coli* cell lysate, respectively. A serial of diluted protein standards and immunoprecipitated products were analyzed by using ONE-HOUR IP-Western Kit (L00232) and THE™ His Tag Antibody, mAb, Mouse (A00186). It was shown that 10 µl (0.05 mg) of the MagBeads can pull out about 25 ng of the 35 kDa His-tagged protein.

XI. LOT-TO-LOT CONSISTENCY

THE™ His Tag Antibody, mAb, Mouse is generated from standard processes and has undergone rigorous quality control tests. This leads to unparalleled lot-to-lot consistency, which saves time and money in optimization of experiment conditions.

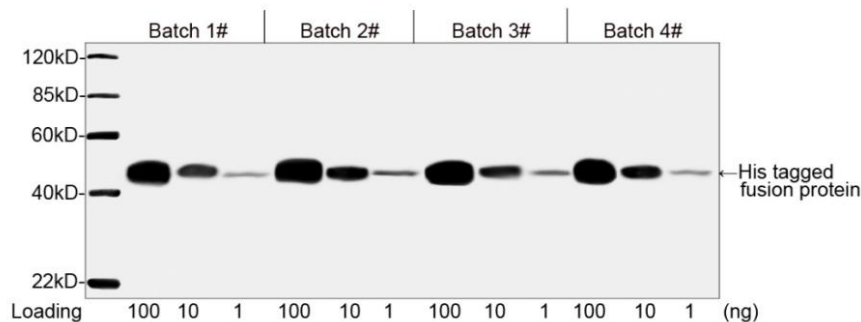


Figure 13. Western blot analysis of four different batches of THE™ His Antibody, mAb, Mouse for lot-to-lot consistency.

XII. TROUBLESHOOTING

Use the table below to solve and avoid common problems in performing a Western blot.

Problem	Probable Cause	Solution
The signal is weak or invisible.	There is too little protein in samples.	Load more protein(s) onto SDS-PAGE gel. Optimize the transfer time and/or the electrical current.
There is high background noise and/or non-specific bands on the blot.	The wash time is too short.	Increased washing after the WB binding step can further decrease background.
	The antibody concentration is too high.	Reduce the concentration of antibody.
	The equipment or reagents have become contaminated.	Use a clean container every time you change solutions for the rinse and wash steps. Wear gloves and use clean forceps to handle the membranes.

XIII. RELATED PRODUCTS

Cat. No.	Product Name	Size	Price
A00612	THE™ His Tag Antibody [HRP], mAb, Mouse	100 µg	\$410.00
A00613	THE™ His Tag Antibody [Biotin], mAb, Mouse	100 µg	\$410.00
A01620	THE™ His Tag Antibody [FITC], mAb, Mouse	100 µg	\$410.00
A00174	His-tag Antibody, pAb, Rabbit	100 µg	\$98.00
A00160	Goat Anti-Mouse IgG (H&L) [HRP]	1 mg	\$59.50
L00275	Mouse Anti-His mAb MagBeads	2 ml	\$197.00

L00223	High Affinity Ni-Charged Resin	10 ml	\$80.00
M0100	Multiple Tag Cell Lysate	200 µg	\$71.50
M0101	Multiple Tag (purified)	200 µg	\$98.00
MM0904	Smart His-Tagged Protein Standard	100 Lanes	\$98.00
L00232	ONE-HOUR IP-Western Kit	1 Kit (5 Assays)	\$125.00

XIV. ORDERING INFORMATION

THE™ His Tag Antibody, mAb, Mouse: Cat. No. A00186

For Research Use Only.

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