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

Technical Manual No. TM0243 **Update date 05202015**

I. DESCRIPTION

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

His tag is an amino acid sequence in proteins that consists of at least four histidine (His) residues, often at the N- or C-terminus of the protein. His-tagged proteins can be produced in different expression systems such as bacteria, yeast and mammalian cells. His-tagged proteins allow for purification by single step chromatography with His tag efficiently coupled to Ni^{2+} affinity resins. His tag monoclonal antibody can be used to identify and detect His-tagged proteins in a variety of immunoassays.

GenScript THE™ His Tag Antibody, mAb, Mouse is produced from the hybridoma that results from fusion of Sp2/0 myeloma and B-lymphocytes obtained from mouse immunized with synthetic peptide HHHHHH coupled to KLH. The antibody is produced from mouse ascites and purified by Protein A affinity column.

II. KEY FEATURES

Features	Specifications
Sensitivity	Less than 5 ng His-tagged proteins by WB
Specificity	Without non-specific binding of other protein
Widespread Diversity	N-terminal/C-terminal/internal His-tagged proteins 4x His/5x His/6x His-tagged proteins
Long-term Stability	Stable in lyophilized form for at least four years
Lot-to-lot Consistency	Guaranteed by strict standard operation

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

IV. 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 (WB): 0.1-0.2 µg/ml
- Dot Blot (DB): user-optimized
- Immunoprecipitation (IP): 1 µg/ml
- Immunofluorescent staining (ICC/IF): 1 µg/ml
- Flow Cytometry (FACS): 1 µg/ml
- TR-FRET assays: user-optimized
- Other applications: user-optimized

V. EXAMPLES

- **ELISA**

THE™ His Tag Antibody, mAb, Mouse was specifically confirmed to recognize different lengths of His peptides by ELISA.

Forms	Peptide sequence	Reactivity
4 x His	HHHH	+
5 x His	HHHHH	+++
6 x His	HHHHHH	+++

- **Western Blot**

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

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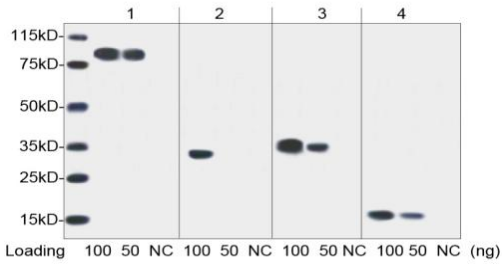
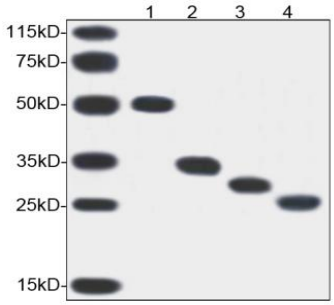
Figure 1. Western blot analysis of 6x His-tagged proteins



	Protein	Tag	Sequence
1	Pfu	C-terminal	……NIKKSGSSHHHHHH
2	T4 kinase	C-terminal	……ASGNFGSSHHHHHH
3	Cart	N-terminal	MSGSHHHHHHSSGMS……
4	Trx	Internal	……GSGSGHMHHHHHHSSGLVP……

Figure 2. Western blot analysis of 4 x His and 5 x His-tagged proteins



	Protein	Tag	Sequence
1	GSTHHHHHGST	Internal	……GHTGHRSHHHHHMSPILG……
2	HHHHHGST	N-terminal	HHHHHSSGMSPILGY……
3	GSTHHHHH	C-terminal	……GHTGHRSGTHHHHH
4	GSTHHHH	C-terminal	…..GHTGHRSGTHHHH

Sensitivity and specificity comparison of THE™ His Tag Antibody, mAb, Mouse (A00186) from GenScript with commercial anti His mAbs from anonymous competitors.

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Figure 3. Western blot analysis of N-terminal His-tagged protein
A: THE™ His Antibody, mAb, Mouse (GenScript, A00186, 0.1 μg/ml)
B: Mouse Anti-His mAb (Competitor A, 0.1 μg/ml)

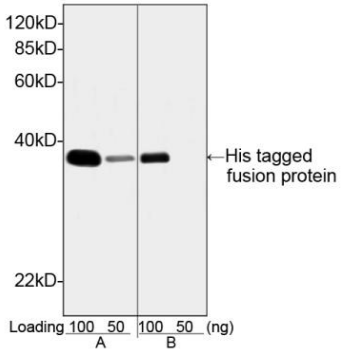


Figure 4. Western blot analysis of C-terminal His-tagged protein
A: THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 μg/ml)
B: Mouse Anti-His mAb (Competitor B, 1 μg/ml)

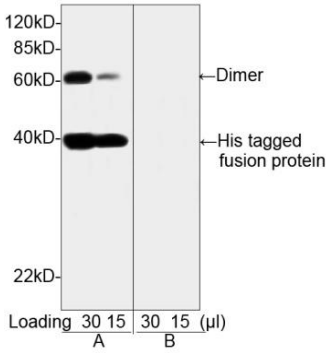
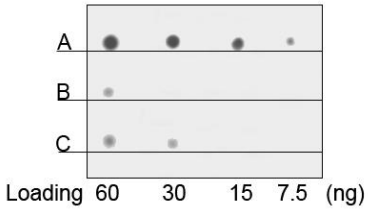


Figure 5. Dot blot analysis of N-terminal 5 x His-tagged protein
A: THE™ His Antibody, mAb, Mouse (GenScript, A00186, 1 μg/ml)
B: Mouse Anti-His mAbs (Competitor Q#1, 1 μg/ml)
C: Mouse Anti-His mAbs (Competitor Q#2, 1 μg/ml)



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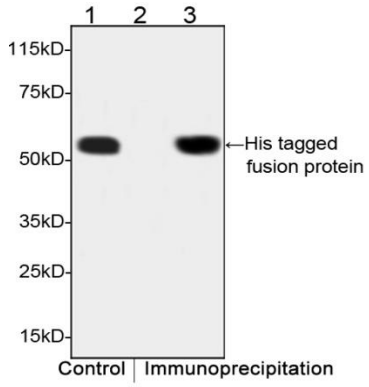
- **Immunoprecipitation**

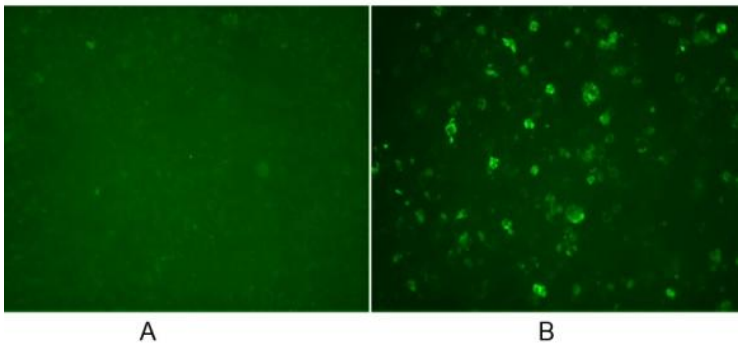
Figure 6. Western blot analysis of immunoprecipitates from cell lysates containing His- tagged protein.

1. Cell lysates containing His-tagged protein
2. Immunoprecipitated cell lysates with isotype control antibody (A01007)
3. Immunoprecipitated cell lysates with THE™ His Tag Antibody, mAb, Mouse (A00186)



- **Immunocytochemistry/ Immunofluorescence**

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)



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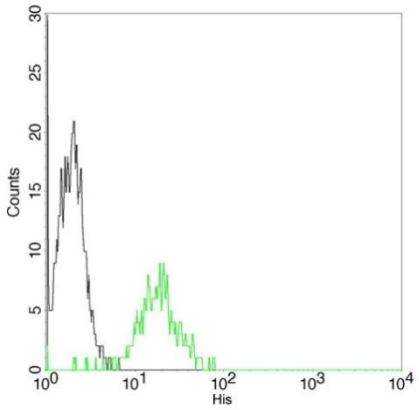
- **FACS**

Figure 8. Flow cytometric analysis of non-transfected or His fusion gene transfeced 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.



VI. 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 experimental conditions.

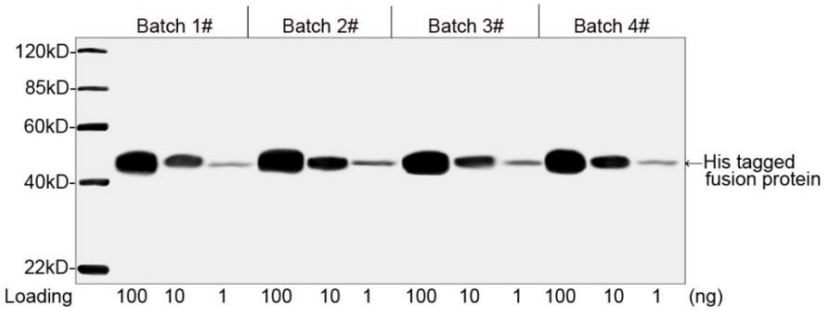


Figure 9. Western blot analysis of four different batches of THE™ His Antibody, mAb, Mouse for lot-to-lot consistency.

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VII. HIS ANTIBODY PRODUCT PIPELINE

His Antibody Product Pipeline is a His tag related product group that can be suitable for different applications. The product is individual, professional and of high quality that can be used for identification, purification and functional analysis of His-tagged proteins.

- His Tag ELISA Detection Kit is used for rapid and high throughput detection of His-tagged proteins.
- His Tag Antibody Plate is used for rapid capture of His-tagged proteins in samples.
- His-Tag Mini Purification and Detection Kit is used for rapid purification and detection of His-tagged proteins

Application	Conjugate	Product Name	Cat. NO
WB, ICC/IF, IP	None	His-tag Antibody, pAb, Rabbit	A00174
WB, ICC/IF, IP	None	THE™ His Tag Antibody, mAb, Mouse	A00186
WB	HRP	THE™ His Tag Antibody [HRP], mAb, Mouse	A00612
WB	Biotin	THE™ His Tag Antibody [Biotin], mAb, Mouse	A00613
ICC/IF, FACS	FITC	THE™ His Tag Antibody [FITC], mAb, Mouse	A01620
ICC/IF, FACS	iFluor 488	THE™ His Tag Antibody [iFluor 488], mAb, Mouse	A01800
ICC/IF	iFluor 555	THE™ His Tag Antibody [iFluor 555], mAb, Mouse	A01801
ICC/IF, FACS	iFluor 647	THE™ His Tag Antibody [iFluor 647], mAb, Mouse	A01802
Purification	Ni-Resin	High Affinity Ni-Charged Resin	L00223
Purification, IP	MagBeads	Mouse Anti-His mAb MagBeads	L00275
ELISA	Kit	His Protein Detection Kit	L00436
Purification	Agarose	Anti-His Affinity Resin	L00439
Capture	Plate	His Tag Antibody Plate	L00440

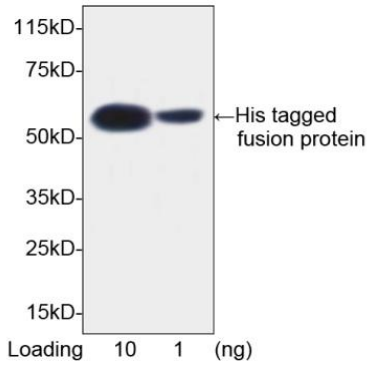

Figure 10. Western blot analysis of His-tagged protein using THE™ His Tag Antibody [HRP], mAb, Mouse (GenScript, A00612, 1 µg/ml)

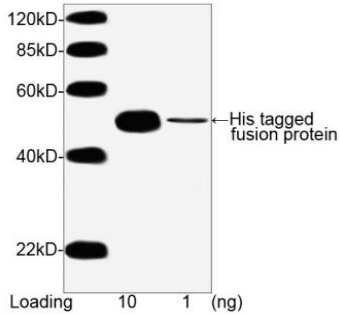

Figure 11. Western blot analysis of His-tagged protein using THE™ His Tag Antibody [Biotin], mAb, mouse (GenScript, A00613, 1 µg/ml)

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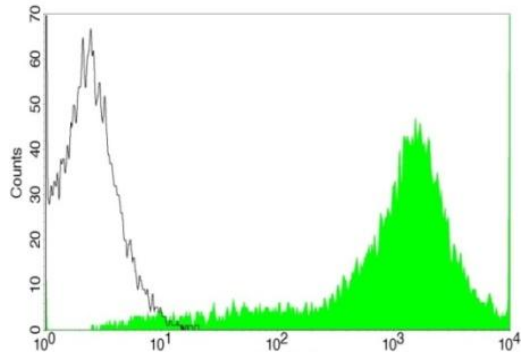

Figure 12. Flow cytometric analysis of HEK293 cells transfected with His-tagged gene using THE™ His Tag Antibody [FITC], mAb, Mouse (GenScript, A01620; shaded histogram) or with a negative control antibody (open histogram).

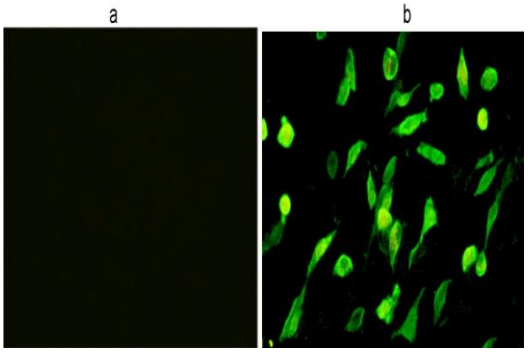

Figure 13. Immunocytochemistry/Immunofluorescence analysis of non-transfected CHO cells (a) or His-tagged protein transfected CHO cells (b) using THE™ His Tag Antibody [iFluor 488], mAb, Mouse

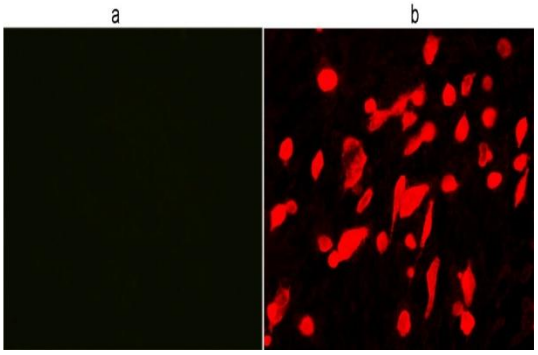

Figure 14.Immunocytochemistry/Immunofluorescence analysis of non-transfected CHO cells (a) and His-tagged protein transfected CHO cells (b) using THE™ His Tag Antibody [iFluor 555], mAb, Mouse GenScript, A01801-100, 4 μg/ml).

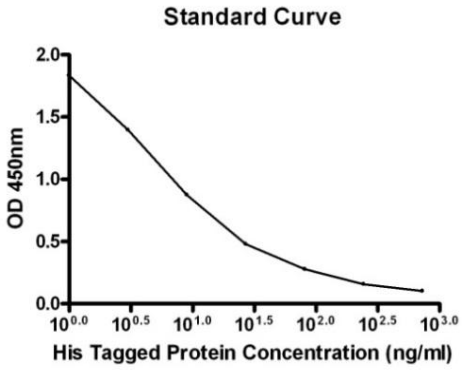

Figure 15. Standard curve of His Tag ELISA Detection Kit (L00436)

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