#12698 Store at -20°C

His-Tag (D3I10) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

☐ Small 100 µl (10 western blots)
☐ Petite 40 µl (4 western blots)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Isotype
W, IP, IF-IC, ChIP, F Transfected	All	Rabbit IgG**

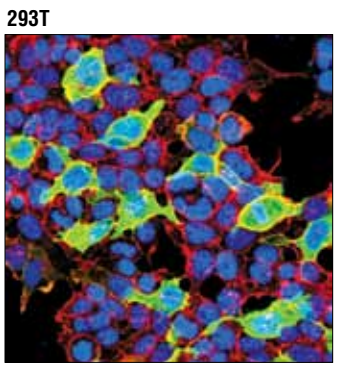
Background: Epitope tags are useful for the labeling and detection of proteins using immunoblotting, immunoprecipitation, and immunostaining techniques. Because of their small size, they are unlikely to affect the tagged protein's biochemical properties.

A variety of plasmids contain DNA that encodes an amino-terminal tag consisting of six histidine (6xHis) residues followed by an extended multiple cloning site. The 6xHis tag on the expressed recombinant proteins allows for efficient coupling to Ni^{2+} affinity resins and purification by single step chromatography (1).

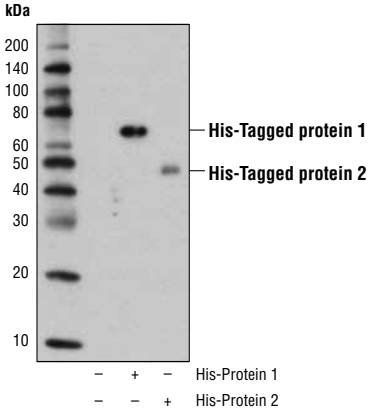
As is the case with other protein tag systems (2), this polyhistidine tag can often be cleaved at sites recognized by proteases such as thrombin and enterokinases to isolate the protein of interest (1).

Specificity/Sensitivity: His-Tag (D3I10) XP® Rabbit mAb recognizes recombinant proteins containing the 6xHis epitope tag. The antibody recognizes the 6xHis-tag fused to either the amino or carboxy terminus of targeted proteins in transfected cells.

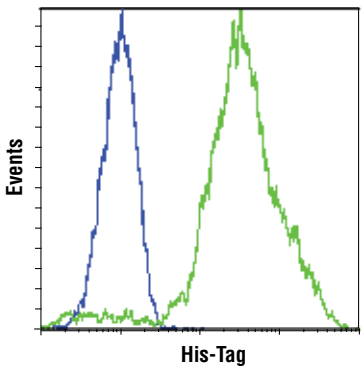
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues of the 6xHis epitope tag.



Confocal immunofluorescent analysis of 293T cells transfected with a His-Tagged protein using His-Tag (D3I10) XP® Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).



Western blot analysis of extracts from 293T cells, untransfected or transfected with 6xHis-Tag fusion protein 1 or 6xHis-Tag fusion protein 2, using His-Tag (D3I10) XP® Rabbit mAb.



Flow cytometric analysis of 293T cells, untransfected (blue) or transfected with a His-myc-Akt plasmid (green), using His-Tag (D3I10) XP® Rabbit mAb. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:400
Chromatin IP	1:100
Flow Cytometry	1:800

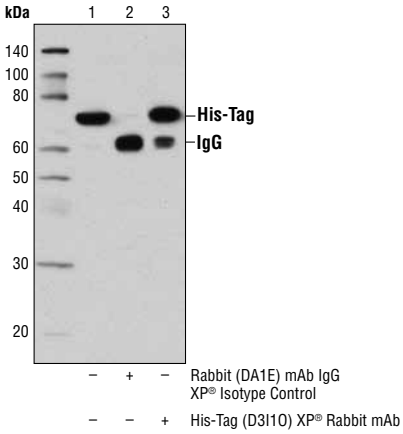
For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Kroll, D.J. et al. (1993) *DNA Cell Biol* 12, 441-53.

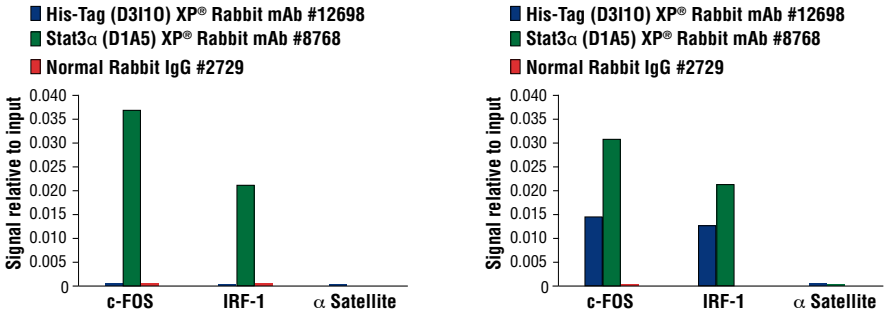
(2) di Guan, C. et al. (1988) *Gene* 67, 21-30.



Immunoprecipitation of His-Tag protein from transfected 293T cells using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or His-Tag (D3I10) XP® Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using His-Tag (D3I10) XP® Rabbit mAb.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



293T cells were either untransfected (left panel) or transfected with an His-tagged human Stat3 construct (right panel), then treated with Human Interleukin-6 (hIL-6) #8904 (100 ng/ml, 30 min). Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 cells and 5 µl of His-Tag (D3I10) XP® Rabbit mAb, 5 µl of Stat3α (D1A5) XP® Rabbit mAb #8768, or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human c-Fos Promoter Primers #4663, human IRF-1 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.