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-20°C  
**#14793**

# DYKDDDDK Tag (D6W5B) Rabbit mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)



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

Applications	Species Cross-Reactivity	Isotype
W, IP, IHC-P, 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.

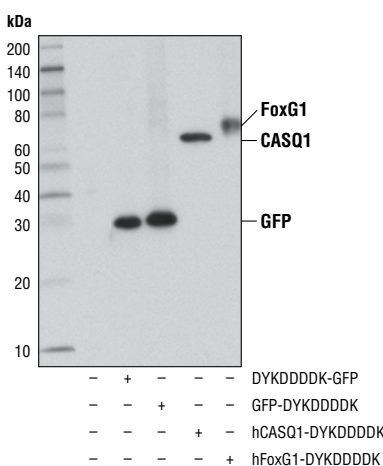
The DYKDDDDK peptide has been used extensively as a general epitope tag in expression vectors. This peptide can be expressed and detected with the protein of interest as an amino-terminal or carboxy-terminal fusion (1).

**Specificity/Sensitivity:** DYKDDDDK Tag (D6W5B) Rabbit mAb detects exogenously expressed DYKDDDDK proteins in cells. The antibody recognizes the DYKDDDDK peptide, which is the same epitope recognized by Sigma's Anti-FLAG® antibodies, fused to either the amino-terminus or carboxy-terminus of the target protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic DYKDDDDK peptide.

**Background References:**

(1) Brizzard, B.L. et al. (1994) *Biotechniques* 16, 730-5.



Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with DYKDDDDK-GFP (N-terminal DDK-Tag; +), GFP-DYKDDDDK (C-terminal DDK-Tag; +), human CASQ1-DYKDDDDK (C-terminal DDK-Tag; +), or human FoxG1-DYKDDDDK (C-terminal DDK-Tag; +) as indicated, using DYKDDDDK Tag (D6W5B) Rabbit mAb.

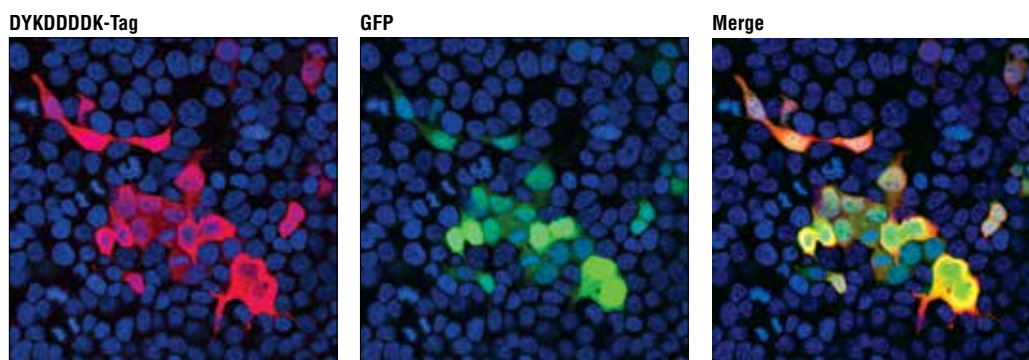
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

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

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:800†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:800
Chromatin IP	1:50
Optimal ChIP conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10 <sup>6</sup> cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.	
Flow Cytometry	1:1600

**For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com)**



Confocal immunofluorescent analysis of 293T cells transfected with a GFP-DYKDDDDK-Tag (green) using DYKDDDDK Tag (D6W5B) Rabbit mAb (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

DRAQ5 is a registered trademark of Biostatus Limited.  
Alexa Fluor is a registered trademark of Life Technologies Corporation.  
Anti-FLAG is a registered trademark of Sigma-Aldrich.  
Tween is a registered trademark of ICI Americas, Inc.

**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.**

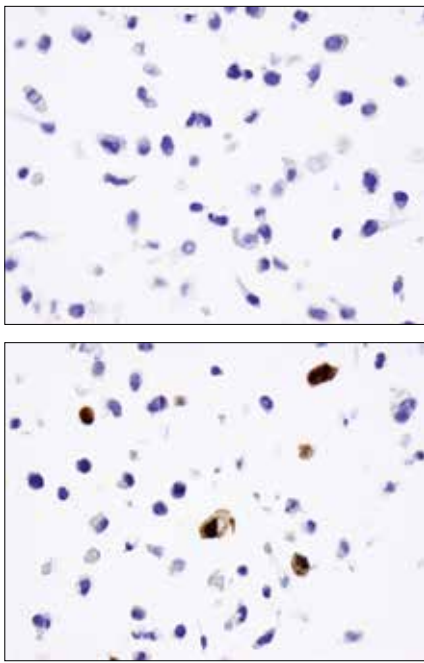
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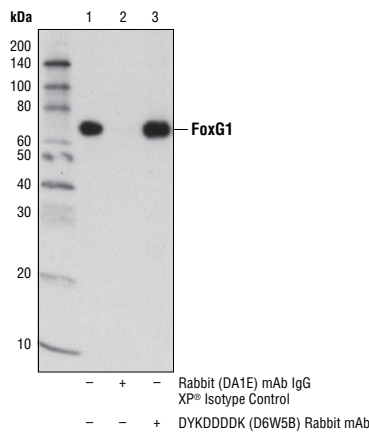
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine 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.

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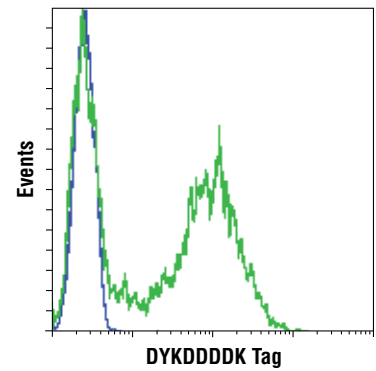
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Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, control (upper) or DYKDDDDK Tag-transfected (lower), using DYKDDDDK Tag (D6W5B) Rabbit mAb.

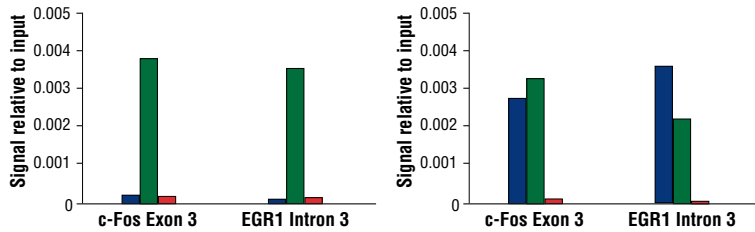


Immunoprecipitation of FoxG1-DYKDDDDK tag protein from 293T transfected cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or DYKDDDDK (D6W5B) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using DYKDDDDK (D6W5B) Rabbit mAb.



Flow cytometric analysis of 293 cells, untransfected (blue) or transfected with DYKDDDDK-Tag (green), using DYKDDDDK Tag (D6W5B) Rabbit mAb. Anti-Rabbit IgG (H+L), F(ab')<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

- DYKDDDDK Tag (D6W5B) Rabbit mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody) #14793
- SSRP1 (E1Y8D) Rabbit mAb #13421
- Normal Rabbit IgG #2729



Chromatin immunoprecipitations were performed with cross-linked chromatin from 293T cells, untransfected (left) or transfected (right) with a DYKDDDDK-tagged SSRP1 construct and DYKDDDDK (D6W5B) Rabbit mAb, SSRP1 (E1Y8D) Rabbit mAb #13421, or 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 Exon 3 Primers #12010 and SimpleChIP® Human EGR1 Intron 3 Primers #11953. 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.

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