DYKDDDK Tag (D6W5B) Rabbit mAb
(Binds to same epitope as Sigma’s Anti-FLAG® M2 Antibody)

Store at -20°C

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

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.

** 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†  
Immunofluorescence (IF-IC) 1:800  
Chromatin IP 1:50  
Flow Cytometry 1:1600

Optimal ChIP conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10⁶ cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

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.

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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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 DYKDDDK 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: DYKDDDK Tag (D6W5B) Rabbit mAb detects exogenously expressed DYKDDDK proteins in cells. The antibody recognizes the DYKDDDK 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 DYKDDDK peptide.

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

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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.

Chromatin immunoprecipitations were performed with cross-linked chromatin from 293T cells, untransfected (left) or transfected (right) with a DYKDDDDK-tagged SSRP1 construct and DYKDDDDK (D6WSB) 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.

Flow cytometric analysis of 293 cells, untransfected (blue) or transfected with DYKDDDDK-Tag (green), using DYKDDDDK Tag (D6WSB) Rabbit mAb. Anti-Rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

Immunoprecipitation of FoxG1-DYKDDDDK tag protein from 293T transfected cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or DYKDDDDK (D6WSB) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using DYKDDDDK (D6WSB) Rabbit mAb.