Revision 4

Store at -20C

#9309

Rb (4H1) Mouse mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IHC-P, IF-IC, FC-FP, ChIP	H Mk B Pg	Endogenous	110	Mouse IgG2a	#P06400	5925

Product Usage Information

For optimal ChIP results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:2000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:800 - 1:3200
Immunofluorescence (Immunocytochemistry)	1:800 - 1:3200
Flow Cytometry (Fixed/Permeabilized)	1:3200
Chromatin IP	1:200

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.

For a carrier-free (BSA and azide free) version of this product see product #61121.

Specificity/Sensitivity

Rb (4H1) Mouse mAb detects endogenous levels of total Rb protein. The antibody does not cross-react with the Rb homologues p107 or p130, or with other proteins.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a Rb-C fusion protein containing residues 701-928 of human Rb. The epitope corresponds to a region surrounding His890 of human Rb.

Background

The retinoblastoma tumor suppressor protein Rb regulates cell proliferation by controlling progression through the restriction point within the G1-phase of the cell cycle (1). Rb has three functionally distinct binding domains and interacts with critical regulatory proteins including the E2F family of transcription factors, c-Abl tyrosine kinase, and proteins with a conserved LXCXE motif (2-4). Cell cycle-dependent phosphorylation by a CDK inhibits Rb target binding and allows cell cycle progression (5). Rb inactivation and subsequent cell cycle progression likely requires an initial phosphorylation by cyclin D-CDK4/6 followed by cyclin E-CDK2 phosphorylation (6). Specificity of different CDK/cyclin complexes has been observed *in vitro* (6-8) and cyclin D1 is required for Ser780 phosphorylation *in vivo* (9).

Background References

1. Sherr, C.J. (1996) *Science* 274, 1672-7.
2. Nevins, J.R. (1992) *Science* 258, 424-9.
3. Welch, P.J. and Wang, J.Y. (1993) *Cell* 75, 779-90.
4. Hu, Q.J. et al. (1990) *EMBO J* 9, 1147-55.
5. Knudsen, E.S. and Wang, J.Y. (1997) *Mol Cell Biol* 17, 5771-83.
6. Lundberg, A.S. and Weinberg, R.A. (1998) *Mol Cell Biol* 18, 753-61.
7. Connell-Crowley, L. et al. (1997) *Mol Biol Cell* 8, 287-301.
8. Kitagawa, M. et al. (1996) *EMBO J* 15, 7060-9.
9. Geng, Y. et al. (2001) *Proc Natl Acad Sci USA* 98, 194-9.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary 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 Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP

Cross-Reactivity Key

H: Human **Mk:** Monkey **B:** Bovine **Pg:** Pig

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