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-Abi tyrosine kinase and proteins with a conserved LXCXE motif (2–4). Cell cycle-dependent phosphorylation by CDK's inhibits Rb target binding, thus allowing cell cycle progression (5). Rb inactivation and subsequent cell cycle progression likely requires first 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).

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.

Recommended Antibody Dilutions:
- Western blotting: 1:2000
- Immunoprecipitation: 1:100
- Immunohistochemistry (Paraffin): 1:800
- Chromatin IP: 1:100
- Immunofluorescence (IF-IC): 1:1600
- Flow Cytometry: 1:400

For application 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:

Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using Rb (4H1) Mouse mAb.

Flow cytometric analysis of Jurkat cells using Rb (4H1) Mouse mAb versus propidium iodide (DNA content). The box indicates Rb positive cells.

Chromatin immunoprecipitations were performed with cross-linked chromatin from Raji cells and either Rb (4H1) Mouse mAb 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 Timeless Intron 1 Primers #7001, human DHFR 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.