

#12231 Store at -20°C

Cyclin B1 (D5C10) XP[®] Rabbit mAb



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

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rev. 01/15/14

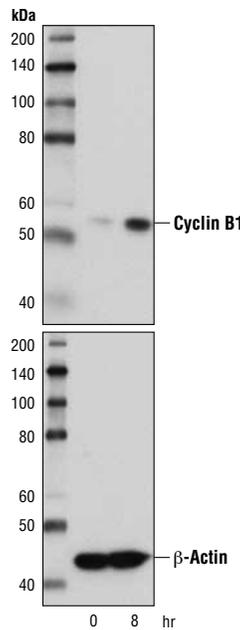
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Applications W, IP, IF-IC, F Endogenous	Species Cross-Reactivity* H, R	Molecular Wt. 55 kDa	Isotype Rabbit IgG**
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Background: Cyclins are a family of proteins that activate specific cyclin-dependent kinases required for progression through the cell cycle. The entry of all eukaryotic cells into mitosis is regulated by activation of cdc2/cdk1 at the G2/M transition. This activation is a multi-step process that begins with the binding of the regulatory subunit, cyclin B1, to cdc2/cdk1 to form the mitosis-promoting factor (MPF). MPF remains in the inactive state until phosphorylation of cdc2/cdk1 at Thr161 by cdk activating kinase (CAK) (1,2) and dephosphorylation of cdc2/cdk1 at Thr14/Tyr15 by cdc25C (3-5). Five cyclin B1 phosphorylation sites (Ser116, 126, 128, 133, and 147) are located in the cytoplasmic retention signal (CRS) domain and are thought to regulate the translocation of cyclin B1 to the nucleus at the G2/M checkpoint, promoting nuclear accumulation and initiation of mitosis (6-9). While MPF itself can phosphorylate Ser126 and Ser128, polo-like kinase 1 (PLK1) phosphorylates cyclin B1 preferentially at Ser133 and possibly at Ser147 (6,10). At the end of mitosis, cyclin B1 is targeted for degradation by the anaphase-promoting complex (APC), allowing for cell cycle progression (11). Research studies have shown that cyclin B1 is overexpressed in breast, prostate, and non-small cell lung cancers (12-14).

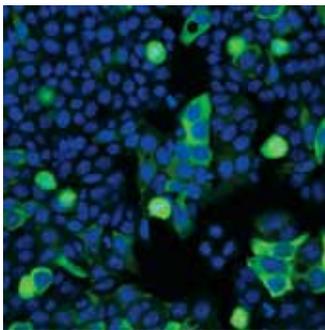
Specificity/Sensitivity: Cyclin B1 (D5C10) XP[®] Rabbit mAb recognizes endogenous levels of total cyclin B1 protein. This antibody also detects a 100 kDa protein of unknown origin in some cell lines.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human cyclin B1 protein.

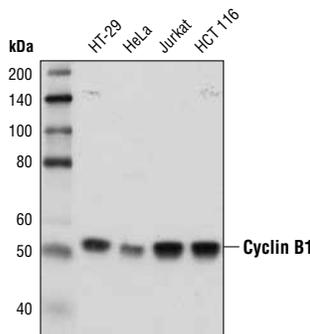


Western blot analysis of extracts from HT-29 cells, synchronized in S-phase by double thymidine block (2 nM, 16 hr) followed by release into fresh media for the indicated time, using Cyclin B1 (D5C10) XP[®] Rabbit mAb (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).

HT-29



Confocal immunofluorescent analysis of HT-29 cells using Cyclin B1 (D5C10) XP[®] Rabbit mAb (green). Blue pseudocolor = DRAQ5[®] #4084 (fluorescent DNA dye).



Western blot analysis of extracts from various cell lines using Cyclin B1 (D5C10) XP[®] Rabbit mAb.

Entrez-Gene ID #891, 5901, 7465
Swiss-Prot Acc. #P14635, P62826, P30291

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	1:100
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:200

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 complementary products.

Background References:

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- (4) McGowan, C.H. and Russell, P. (1993) *EMBO J* 12, 75-85.
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- (6) Toyoshima-Morimoto, F. et al. (2001) *Nature* 410, 215-20.
- (7) Li, J. et al. (1997) *Proc Natl Acad Sci U S A* 94, 502-7.
- (8) Takizawa, C.G. and Morgan, D.O. (2000) *Curr Opin Cell Biol* 12, 658-65.
- (9) Santos, S.D. et al. (2012) *Cell* 149, 1500-13.
- (10) Jackman, M. et al. (2003) *Nat Cell Biol* 5, 143-8.
- (11) Gong, D. and Ferrell, J.E. (2010) *Mol Biol Cell* 21, 3149-61.
- (12) Mashal, R.D. et al. (1996) *Cancer Res* 56, 4159-63.
- (13) Kawamoto, H. et al. (1997) *Am J Pathol* 150, 15-23.
- (14) Soria, J.C. et al. (2000) *Cancer Res* 60, 4000-4.

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

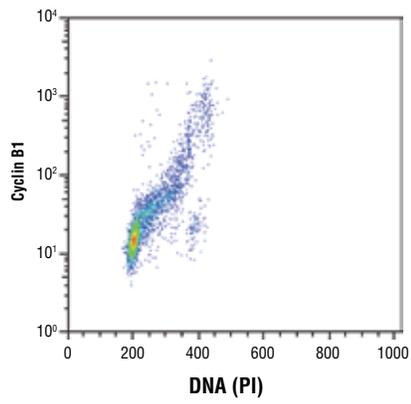
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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink 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.



Flow cytometric analysis of Jurkat cells using Cyclin B1 (D5C10) XP[®] Rabbit mAb and Propidium Iodide/RNase Staining Solution #4087 (DNA content); anti-rabbit IgG (H+L), F(ab')₂ fragment (Alexa Fluor 488 Conjugate) #4412 was used as a secondary Ab.