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Phospho-Cyclin B1 (Ser147) Antibody

Cell Signaling TECHNOLOGY®

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

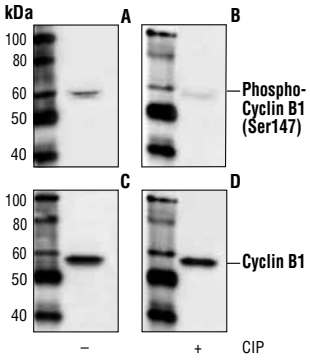
Entrez-Gene ID #891
UniProt Acc. #P14635

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, (Pg)	55 kDa	Rabbit**

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: Phospho-Cyclin B1 (Ser147) Antibody detects endogenous levels of cyclin B1 only when phosphorylated at serine 147.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser147 of human cyclin B1. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 293 cells, using Phospho-Cyclin B1 (Ser147) Antibody (A) and Cyclin B1 (V152) Monoclonal Antibody (C). Treatment of the membrane with calf intestinal alkaline phosphatase (CIP) after Western transfer abolishes the phospho-cyclin B1 signal (B), but has no effect on the total cyclin B1 signal (D).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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

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:

(1) Lorca, T. et al. (1992) *EMBO J* 11, 2381-90.
(2) Harper, J.W. and Elledge, S.J. (1998) *Genes Dev* 12, 285-9.
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(4) McGowan, C.H. and Russell, P. (1993) *EMBO J* 12, 75-85.
(5) Atherton-Fessler, S. et al. (1994) *Mol Biol Cell* 5, 989-1001.
(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.
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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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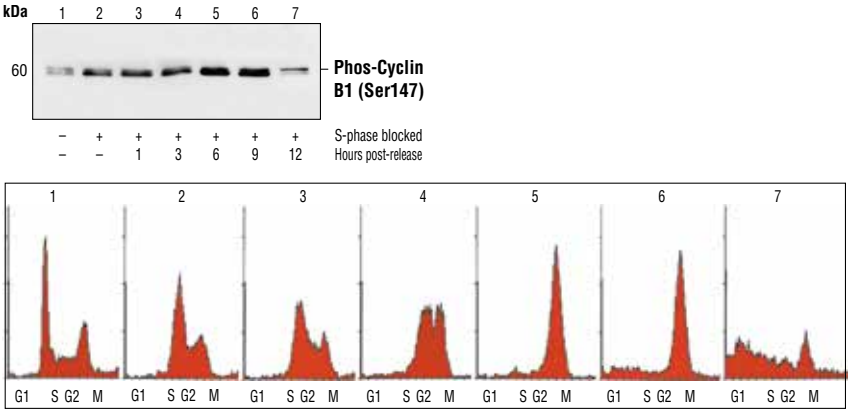
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



Western blot analysis of extracts from asynchronous cells (lane 1), S-phase and G2/M blocked cells (lane 2), or synchronized cells collected at various time points following release from this block (lanes 3-7) using Phospho-Cyclin B1 (Ser147) Antibody (upper). Cell cycle synchronization was verified by flow cytometric analysis of DNA content (lower). (Data kindly provided by Ethan Kohn and Alan Eastman, Dartmouth Medical School, Hanover, NH).