

#3300 Store at -20°C

Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb

✓ 100 µl
(10 western blots)



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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F Endogenous	H, (Mk)	36 kDa	Rabbit IgG**

Background: Activity of the cyclin-dependent kinases CDK4 and CDK6 is regulated by T-loop phosphorylation, by the abundance of their cyclin partners (the D-type cyclins), and by association with CDK inhibitors of the Cip/Kip or INK family of proteins (1). The inactive ternary complex of cyclin D/CDK4 and p27 Kip1 requires extracellular mitogenic stimuli for the release and degradation of p27 concomitant with a rise in cyclin D levels to effect progression through the restriction point and pRb-dependent entry into S-phase (2). The active complex of cyclin D/CDK4 targets the retinoblastoma protein for phosphorylation, allowing the release of E2F transcription factors that activate G1/S-phase gene expression (3). Levels of cyclin D protein drop upon withdrawal of growth factors through downregulation of its protein expression and through phosphorylation-dependent degradation (4).

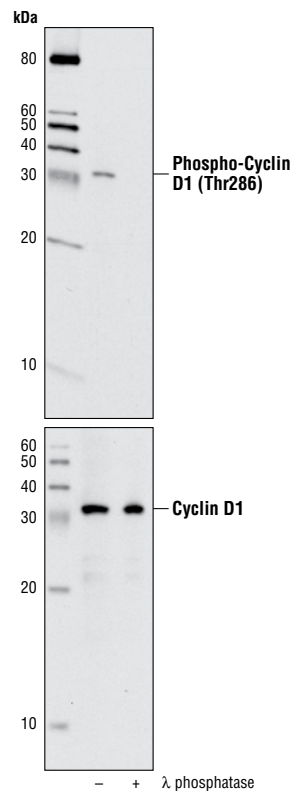
Aberrant expression of cyclin D1 is associated with many forms of cancer, including B cell lymphomas. Gene translocation or amplification of the cyclin D1 gene can directly contribute to oncogenesis (2). Cyclin D1 also plays a critical role in mammary tissue maturation (5). Phosphorylation of cyclin D1 at Thr286 by glycogen synthase kinase 3β (4) or through the Ras/Raf/MEK/MAPK pathway (6) enhances its ubiquitination and proteasomal degradation.

Specificity/Sensitivity: Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb detects endogenous levels of cyclin D1 only when phosphorylated at Thr286. The antibody does not cross-react with other cyclin D family members.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr286 of cyclin D1.

Background References:

- (1) Hirai, H. et al. (1995) *Mol. Cell. Biol.* 15, 2672–2681.
- (2) Sherr, C.J. (1996) *Science* 274, 1672–1677.
- (3) Lukas, J. et al. (1996) *Mol. Cell. Biol.* 16, 6917–6925.
- (4) Diehl, J.A. et al. (1997) *Genes Dev.* 11, 957–972.
- (5) Sicinski, P. et al. (1995) *Cell* 82, 621–630.
- (6) Shao, J. et al. (2000) *J Biol Chem* 275, 22916–24.



Western blot analysis of extracts from HT-1080 cells, treated with MG132 and with or without λ phosphatase, using Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb (upper) or Cyclin D1 (DC56) Mouse mAb #2926 (lower).

Flow cytometric analysis of HT-1080 cells, untreated (blue) or MG132-treated (green), using Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb.

Entrez-Gene ID #595
Swiss-Prot Acc. #P24385

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.

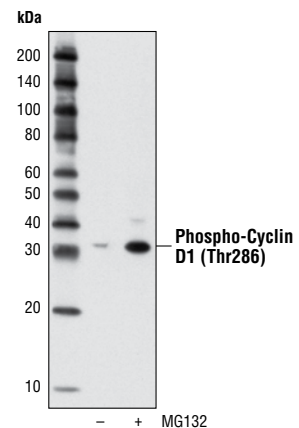
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

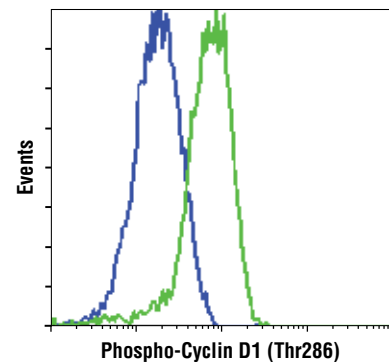
Western blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:1000
Flow Cytometry	1:400

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.

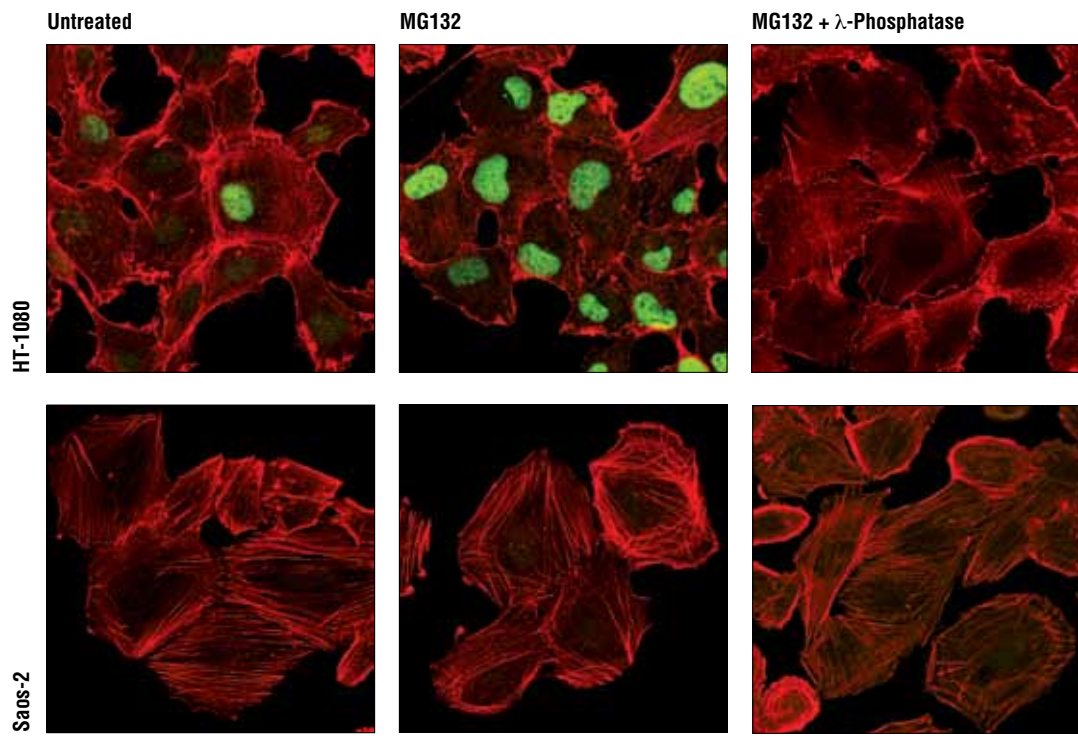


Western blot analysis of extracts from HT-1080 cells, untreated or treated with MG132 (10 µM, 4 hours), using Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb.



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.

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.



Confocal immunofluorescent analysis of HT-1080 (top) and Saos-2 cells (bottom), untreated (left) or treated with MG132 alone (center) or with MG132 followed by λ -phosphatase (right), using Phospho-Cyclin D1 (Thr286) (D29B3) XP™ Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).