Store at –20°C

#2921

Phospho-Cyclin D1 (Thr286) Antibody

☑ 100 µl
(10 western blots)

Cell Signaling TECHNOLOGY®

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, R, (M)	36 kDa	Rabbit**

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).

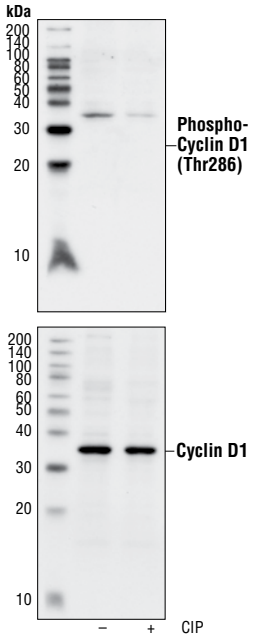
Ubiquitination of cyclin D1 is enhanced by phosphorylation at Thr286 by glycogen synthase kinase 3beta (GSK-3β) (4).

Specificity/Sensitivity: Phospho-Cyclin D1 (Thr286) Antibody detects endogenous levels of cyclin D1 only when phosporylated at threonine 286. The antibody does not cross-react with other cyclin D family members at physiological levels.

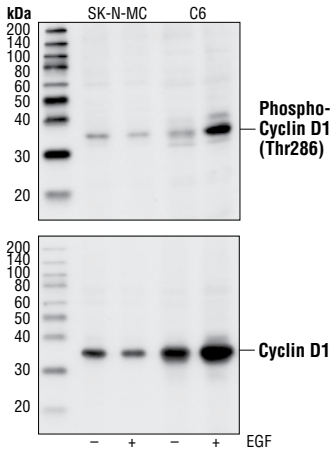
Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr286 of human cyclin D1. Antibodies are purified by protein A and peptide affinity chromatography.

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.



Western blot analysis of extracts from SK-N-MC cells, untreated or calf intestinal phosphatase (CIP)-treated, using Phospho-Cyclin D1 (Thr286) Antibody (upper) or Cyclin D1 Mouse mAb #2926 (lower).



Western blot analysis of extracts from SK-N-MC and C6 cells, untreated or EGF-treated, using Phospho-Cyclin D1 (Thr286) Antibody (upper) or Cyclin D1 mouse mAb #2926 (lower).

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.

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.