#12028 Store at -20°C

Phospho-cdc25C (Thr48) (D2H3) Rabbit mAb

Cell Signaling TECHNOLOGY®

☑ 100 µl
(10 western blots)

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rev. 01/05/15

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Entrez-Gene ID #995
UniProt Acc. #P30307

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC	H, (Mk)	75 kDa	Rabbit IgG**
Endogenous			

Background: Cdc25 is a protein phosphatase responsible for dephosphorylating and activating cdc2, a crucial step in regulating the entry of all eukaryotic cells into mitosis (1). cdc25C is constitutively phosphorylated at Ser216 throughout interphase by c-TAK1, while phosphorylation at this site is DNA damage-dependent at the G2/M checkpoint (2). When phosphorylated at Ser216, cdc25C binds to members of the 14-3-3 family of proteins, sequestering cdc25C in the cytoplasm and thereby preventing premature mitosis (3). The checkpoint kinases Chk1 and Chk2 phosphorylate cdc25C at Ser216 in response to DNA damage (4,5).

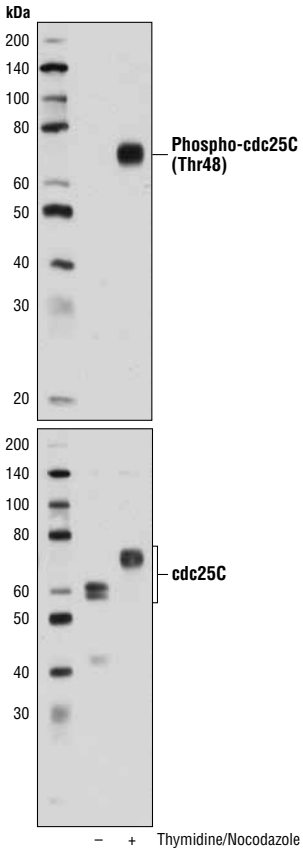
Full activation of cdc25C involves phosphorylation at more than 12 different sites by cdc2/cyclin B and Polo-like kinase, and the activity of Pin1, a peptidyl-prolyl isomerase (PPI) (6,7). Pin1 contains a WW domain that binds phospho-Ser/Thr-Pro sites and a catalytic PPI region that induces a cis/trans isomerization at phospho-Ser/Thr-Pro bonds (8). Thr48 and Thr67 of cdc25C interact directly with the WW domain of Pin1 when these sites are phosphorylated (9). Thr48 phosphorylation also mediates binding to CKS/p13SUC1 (10).

Specificity/Sensitivity: Phospho-cdc25C (Thr48) (D2H3) Rabbit mAb recognizes endogenous levels of cdc25C protein only when phosphorylated at Thr48.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr48 of human cdc25C protein.

Background References:

(1) Jessus, C. and Ozon, R. (1995) *Prog. Cell Cycle Res.* 1, 215-228.
(2) Peng, C.Y. et al. (1997) *Science* 277, 1501-1505.
(3) Kumagai, A. and Dunphy, W.G. (1999) *Genes Dev.* 13, 1067-1072.
(4) Blasina, A. et al. (1999) *Curr. Biol.* 9, 1-10.
(5) Furnari, B. et al. (1999) *Mol. Biol. Cell* 10, 833-845.
(6) Izumi, T. and Maller, J.L. (1993) *Mol Biol Cell* 4, 1337-50.
(7) Stukenberg, P.T. and Kirschner, M.W. (2001) *Mol Cell* 7, 1071-83.
(8) Yaffe, M.B. et al. (1997) *Science* 278, 1957-60.
(9) Lu, P.J. et al. (1999) *Science* 283, 1325-8.
(10) Landrieu, I. et al. (2001) *J Biol Chem* 276, 1434-8.



Western blot analysis of extracts from HT-29 cells, asynchronous (-) or synchronized in mitosis (thymidine block, nocodazole release, mitotic shake-off; +), using Phospho-cdc25C (Thr48) (D2H3) Rabbit mAb (upper) or cdc25C (5H9) Rabbit mAb #4688 (lower).

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.

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.**

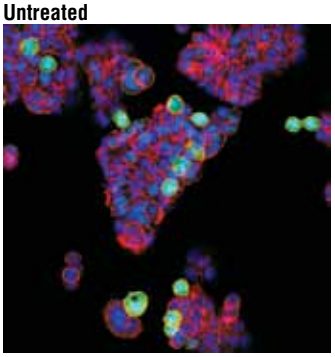
Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:300

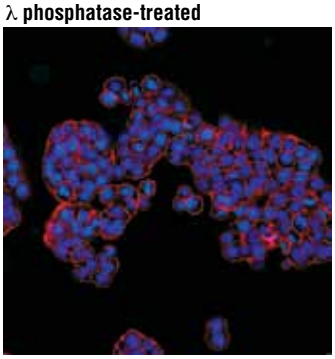
For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Untreated



λ phosphatase-treated



Confocal immunofluorescent analysis of HT-29 cells, untreated (upper) or λ phosphatase-treated (lower), using Phospho-cdc25c (Thr48) (D2H3) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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.