Store at -20°C

#70307

Phospho-CAD (Ser1859) (D5O6C) Rabbit mAb

Cell Signaling TECHNOLOGY®

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New 05/17

Entrez-Gene ID #790
UniProt ID #P27708

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC	H, M, R	240 kDa	Rabbit IgG**
Endogenous			

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
Immunofluorescence (IF-IC)	1:50

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background: CAD is essential for the *de novo* synthesis of pyrimidine nucleotides and possesses the following enzymatic activities: glutamine amidotransferase, carbamoyl-phosphate synthetase, aspartate transcarbamoylase, and dihydroorotase. Thus, the enzyme converts glutamine to uridine monophosphate, a common precursor of all pyrimidine bases, and it is necessary for nucleic acid synthesis (1). In resting cells, CAD is localized mainly in the cytoplasm where it carries out pyrimidine synthesis. As proliferating cells enter S phase, MAP Kinase (Erk1/2) phosphorylates CAD at Thr456, resulting in CAD translocation to the nucleus. As cells exit S phase, CAD is dephosphorylated at Thr456 and phosphorylated at Ser1406 by PKA, returning the pathway to basal activity (2). Various research studies have shown increased expression of CAD in several types of cancer, prompting the development of pharmacological inhibitors such as PALA. Further studies have identified CAD as a potential predictive early marker of prostate cancer relapse (3). mTORC1 is a protein kinase that works to regulate the growth and proliferation of cells by sensing and integrating various growth signals. S6 kinase 1 (S6K1) is a downstream ribosomal protein target of mTORC1 and directly phosphorylates Ser1859 on CAD. This phosphorylation stimulates the first three steps of the *de novo* primidine synthesis and thus helps to advance the cells' overall progression through S phase of the cell cycle (4,5).

Background References:

(1) Coleman, P.F. et al. (1977) *J Biol Chem* 252, 6379-85.

(2) Sigoillot, F.D. et al. (2005) *J Biol Chem* 280, 25611-20.

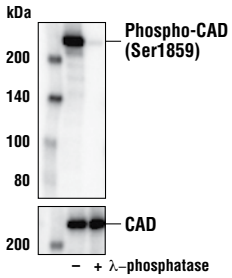
(3) Morin, A. et al. (2012) *FASEB J* 26, 460-7.

(4) Ben-Sahra, I. et al. (2013) *Science* 339, 1323-8.

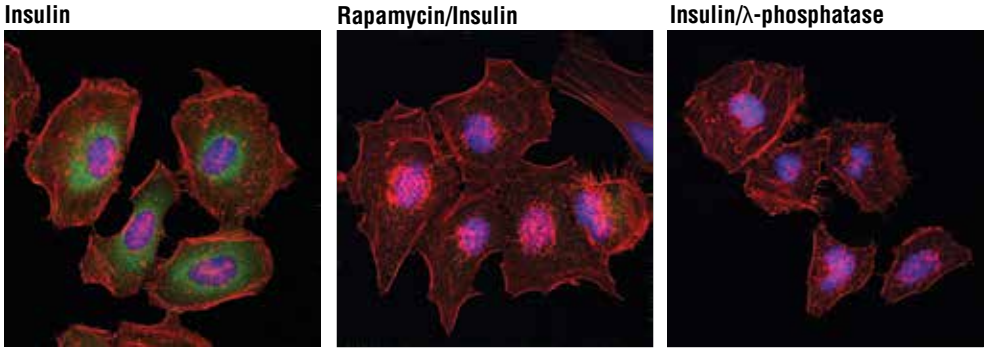
(5) Robitaille, A.M. et al. (2013) *Science* 339, 1320-3.

Specificity/Sensitivity: Phospho-CAD (Ser1859) (D5O6C) Rabbit mAb recognizes endogenous levels of CAD protein only when phosphorylated at Ser1859.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser1859 of human CAD protein.



Western blot analysis of extracts from 293T cells, untreated (-) or λ-phosphatase-treated (+), using Phospho-CAD (Ser1859) (D5O6C) Rabbit mAb (upper) and CAD Antibody #11933 (lower).



Confocal immunofluorescent analysis of insulin-treated (100 nM, 1 hr) HeLa cells, either pretreated with Rapamycin #9904 (20 nM, 1 hr; center) or post-processed with λ-phosphatase (right), using Phospho-CAD (Ser1859) (D506C) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.