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#14717

Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb

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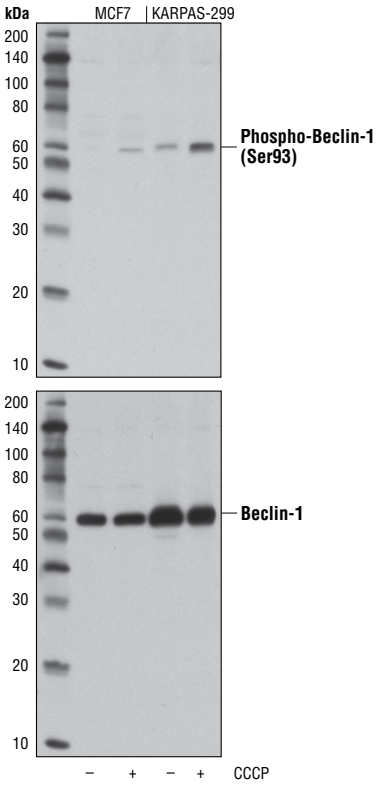
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP Endogenous	H, (M, R, B, Dg, Pg)	60 kDa	Rabbit IgG**

Background: Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of proteins activated in response to nutrient deprivation and in neurodegenerative conditions (1). One of the proteins critical to this process is Beclin-1, the mammalian orthologue of the yeast autophagy protein Apg6/Vps30 (2). Beclin-1 can complement defects in yeast autophagy caused by loss of Apg6 and can also stimulate autophagy when overexpressed in mammalian cells (3). Mammalian Beclin-1 was originally isolated in a yeast two-hybrid screen for Bcl-2 interacting proteins and has been shown to interact with Bcl-2 and Bcl-xL, but not with Bax or Bak (4). While Beclin-1 is generally ubiquitously expressed, research studies have shown it is monoallelically deleted in 40-75% of sporadic human breast and ovarian cancers (5). Beclin-1 is localized within cytoplasmic structures including the mitochondria, although overexpression of Beclin-1 reveals some nuclear staining and CRM1-dependent nuclear export (6). Investigators have demonstrated that Beclin-1 -/- mice die early in embryogenesis and Beclin-1 -/+ mice have a high incidence of spontaneous tumors. Stem cells from the null mice demonstrate an altered autophagic response, although responses to apoptosis appeared normal (7). Researchers have also found that overexpression of Beclin-1 in virally infected neurons *in vivo* resulted in significant protection against Sindbis virus-induced disease and neuronal apoptosis (4).

To induce autophagy, AMP-activated protein kinase (AMPK) directly phosphorylates Beclin-1 at conserved Ser93 and Ser96 residues in human, which correspond to murine Ser91 and Ser94 (8).

Specificity/Sensitivity: Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb recognizes endogenous levels of Beclin-1 protein only when phosphorylated at Ser93. This antibody recognizes single phosphorylation of Ser93 to a greater extent than dual phosphorylation at Ser93 and Ser96. This antibody may also weakly detect an unidentified band at approximately 70 kDa.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser93 of human Beclin-1 protein (Ser91 in mouse).



Western blot analysis of extracts from MCF7 and KARPAS-299 cells, untreated (-) or treated with carbonyl cyanide 3-chlorophenylhdrazone (CCCP, 100 µM, 2 hr; +), using Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb (upper) and Beclin-1 (D40C5) Rabbit mAb #3495 (lower). Cell line source: Dr Abraham Karpas at the University of Cambridge.

Immunoprecipitation of phospho-Beclin-1 (Ser93) from carbonyl cyanide 3-chlorophenyldydrazone (CCCP)-treated KARPAS-299 cells (100 µM, 2 hr) using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot was performed using Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb #3678 was used as a secondary antibody to avoid cross reactivity with IgG heavy chain. Cell line source: Dr Abraham Karpas at the University of Cambridge.

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	1:50

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

Background References:

(1) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot Cell* 1, 11-21.

(2) Kametaka, S. et al. (1998) *J Biol Chem* 273, 22284-91.

(3) Liang, X.H. et al. (1999) *Nature* 402, 672-6.

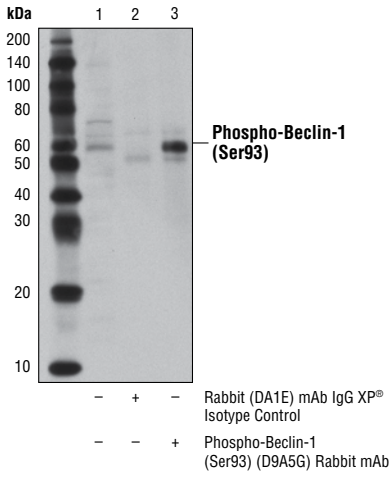
(4) Liang, X.H. et al. (1998) *J Virol* 72, 8586-96.

(5) Aita, V.M. et al. (1999) *Genomics* 59, 59-65.

(6) Liang, X.H. et al. (2001) *Cancer Res* 61, 3443-9.

(7) Yue, Z. et al. (2003) *Proc Natl Acad Sci USA* 100, 15077-82.

(8) Kim, J. et al. (2013) *Cell* 152, 290-303.



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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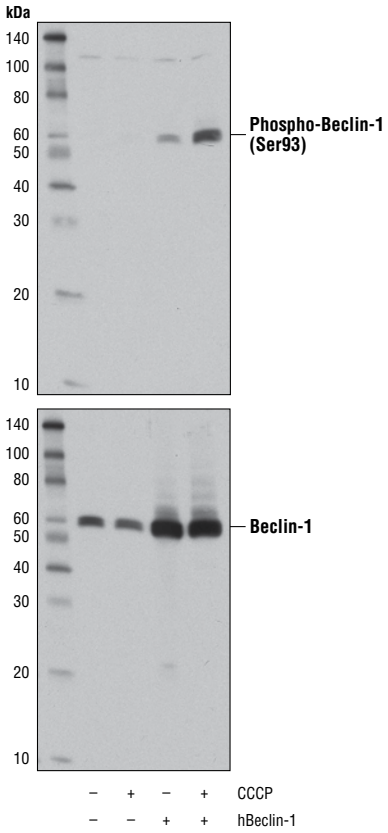
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



Western blot analysis of extracts from 293T cells, untreated (-) or treated with carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 100 µM, 2 hr; +) and mock transfected (-) or transfected with a construct expressing full-length human Beclin-1 (hBeclin-1; +), using Phospho-Beclin-1 (Ser93) (D9A5G) Rabbit mAb (upper) and Beclin-1 (D40C5) Rabbit mAb #3495 (lower).