

Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb



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Applications W, IP, F Endogenous	Species Cross-Reactivity* H, M, R, Mk, (C, X, Z, B, Dg, Pg, Hr)	Molecular Wt. 90 kDa	Isotype Rabbit IgG**
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Background: The 90 kDa ribosomal S6 kinases (RSK1-4) are a family of widely expressed serine/threonine kinases characterized by two nonidentical, functional kinase domains (1) and a carboxy-terminal docking site for extracellular signal-regulated kinases (ERKs) (2). Several sites both within and outside of the RSK kinase domain, including Ser380, Thr359, Ser363, and Thr573, are important for kinase activation (3). RSK1-3 are activated via coordinated phosphorylation by MAPKs, autophosphorylation, and phosphoinositide-3-OH kinase (PI3K) in response to many growth factors, polypeptide hormones, and neurotransmitters (3).

Upon mitogenic stimulation, p44/42 ERK1/2 and ERK5 MAP kinases cooperatively phosphorylate p90RSK Thr573 (p90RSK1 numbering) located within the C-terminal kinase domain and Thr359/Ser363 in the linker region between the two kinase domains (3). Phosphorylation of Thr573 within the activation loop of the p90RSK C-terminal kinase domain promotes activation and directs phosphorylation of Ser380 within the hydrophobic stretch of the linker region (4,5). The phosphorylated p90RSK Ser380 acts as a docking site for

the constitutively active Ser/Thr kinase PDK1, which in turn phosphorylates Ser221 within the N-terminal kinase domain activation loop, resulting in full enzymatic activation of the p90RSK (6). Antibodies against these phosphorylation sites are useful for understanding the kinetics and regulation of p90RSK activation.

For more information regarding the phospho-regulatory sites within each RSK isoform, including more information regarding the seminal studies demonstrating the complex phosphorylation cascades involved, please see the references herein and PhosphoSitePlus® (www.phosphosite.org).

Specificity/Sensitivity: Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb recognizes endogenous levels of RSK1, RSK2 and RSK3 proteins only when phosphorylated at Ser380 (RSK1), Ser386 (RSK2), or Ser377 (RSK3).

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser377 of human p90RSK3 protein.

Entrez-Gene ID #6195, 6196, 6197
UniProt Acc. #Q15418, Q15349, P51812

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.**

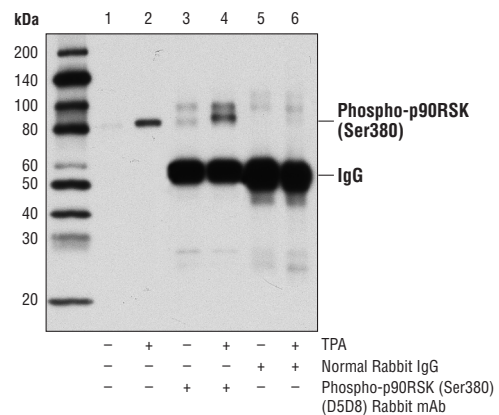
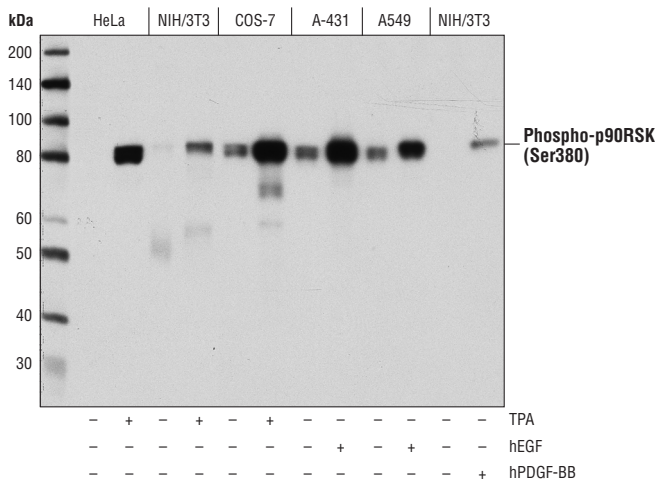
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Flow Cytometry	1:400

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.



Western blot analysis of extracts from various cell lines, starved overnight and untreated (-) or treated with either TPA #4174 (200 nM, 15 min; +), hEGF #8916 (100 ng/ml, 15 min; +), or hPDGF-BB #8912 (100 ng/ml, 15 min; +), using Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb.

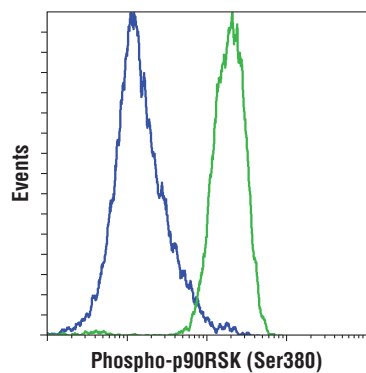
Immunoprecipitation of p90RSK from extracts of HeLa cells serum starved overnight, untreated (-) or treated with TPA #4174 (200 nM, 15 min; +), using Normal Rabbit IgG #2729 (lanes 5 and 6) or Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb (lanes 3 and 4). Lanes 1 and 2 are 10% input. Western blot analysis was performed using Phospho-p90RSK (Ser380) Antibody #9341.

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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.



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with TPA #4174 (green), using Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb. Anti-rabbit IgG (H+L), F(ab)₂ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody.

Background References:

- (1) Fisher, T.L. and Blenis, J. (1996) *Mol Cell Biol* 16, 1212-9.
- (2) Smith, J.A. et al. (1999) *J Biol Chem* 274, 2893-8.
- (3) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496-505.
- (4) Roux, P.P. et al. (2003) *Mol Cell Biol* 23, 4796-804.
- (5) Cargnello, M. and Roux, P.P. (2011) *Microbiol Mol Biol Rev* 75, 50-83.
- (6) Romeo, Y. et al. (2012) *Biochem J* 441, 553-69.