Store at –20°C

#2281

SPAK Antibody

☑ 100 µl
(10 western blots)

Cell Signaling TECHNOLOGY®

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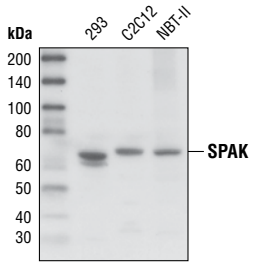
rev. 1/15/10

Entrez-Gene ID #27347
Swiss-Prot Acc. #Q9UEW8

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-F	H, M, R, Mk	65 kDa	Rabbit**
Endogenous			

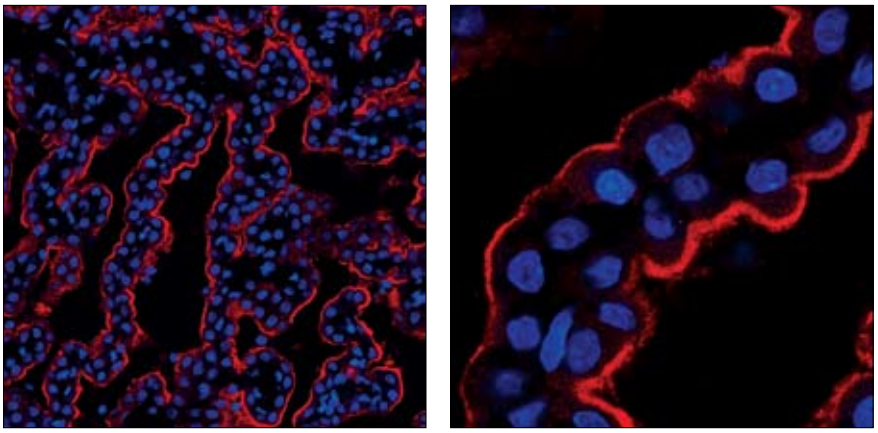
Background: SPAK (STE20/SPS1-related Pro/Ala-rich kinase) and OSR1 (oxidative stress responsive 1) are members of the GCK family serine/threonine kinases. Overexpression and *in vitro* studies demonstrate SPAK is able to activate p38 MAP kinase, which indicates SPAK might be a mediator of stress response (1). Yeast two-hybrid screening revealed that SPAK and OSR1 bind to Na-K-2CL cotransporters NKCC1 and 2 and K-CL cotransporter KCC3 (2). WNK1 and WNK4 phosphorylate SPAK at Thr243/247 and Ser380 (3–5). Similarly, WNK1 and WNK4 phosphorylate OSR1 at Thr185 and Ser315 (3,4). Phosphorylation at these sites stimulates SPAK and OSR1 activity, leading to NKCC1 phosphorylation and enhanced NKCC1 activity (3–5). SPAK is also phosphorylated at Ser311 by PKCθ in response to T cell activation. Substitution of Ser311 with Ala or specific siRNA knock-down of SPAK dramatically reduces TCR/CD28-induced AP-1 activation, suggesting SPAK is involved in T cell signaling as well (6).

Specificity/Sensitivity: SPAK Antibody detects endogenous levels of total SPAK protein. This antibody does not cross-react with OSR1 or other members of the GCK family.



Western blot analysis of extracts from 293, C2C12, and NBTII cells, using SPAK Antibody.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Ser436 of human SPAK. Antibodies are purified by protein A and peptide affinity chromatography.



Confocal immunofluorescent analysis of choroid plexus epithelial cells showing apical membrane localization, using SPAK Antibody (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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 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
Immunoprecipitation	1:100
Immunofluorescence (IF-F)	1:50

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.

Background References:

(1) Johnston, A.M. et al. (2000) *Oncogene* 19, 4290–4297.
(2) Piechotta, K. et al. (2002) *J. Biol. Chem.* 277, 50812–50819.
(3) Vitari, A.C. et al. (2005) *Biochem J.* 391, 17–24.
(4) Moriguchi, T. et al. (2005) *J. Biol. Chem.* 280, 42685–42693.
(5) Gagnon, K.B. et al. (2006) *Mol. Cell. Biol.* 26, 689–698.
(6) Li, Y. et al. (2004) *EMBO J.* 23, 1112–1122.

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.