Revision 6

Store at -20C

#2281

SPAK Antibody

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, IF-F	H M R Mk	Endogenous	65	Rabbit	#Q9UEW8	27347

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Frozen)	1:50

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.

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.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser436 of human SPAK. Antibodies are purified by protein A and peptide affinity chromatography.

Background

SPAK (STE20/SPS1-related Pro/Ala-rich kinase) and OSR1 (oxidative stress responsive 1) are members of the GCK family of serine/threonine kinases. Overexpression and *in vitro* studies demonstrate that SPAK is able to activate p38 MAP kinase, indicating a possible role for SPAK in the stress response (1). Yeast two-hybrid screening revealed that SPAK and OSR1 bind to Na-K-2Cl cotransporters NKCC1 and NKCC2 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 knockdown of SPAK dramatically reduces TCR/CD28-induced AP-1 activation, suggesting SPAK is involved in T cell signaling as well (6).

Background References

1. Johnston, A.M. et al. (2000) *Oncogene* 19, 4290-7.
2. Piechotta, K. et al. (2002) *J Biol Chem* 277, 50812-9.
3. Vitari, A.C. et al. (2005) *Biochem J* 391, 17-24.
4. Moriguchi, T. et al. (2005) *J Biol Chem* 280, 42685-93.
5. Gagnon, K.B. et al. (2006) *Mol Cell Biol* 26, 689-98.
6. Li, Y. et al. (2004) *EMBO J* 23, 1112-22.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IF-F:** Immunofluorescence (Frozen)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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Limited Uses

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