

## **SPAK Antibody (Center)**

Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP7968c

# **Specification**

# **SPAK Antibody (Center) - Product Information**

Application WB, IHC-P,E
Primary Accession Q9UEW8

Other Accession
Reactivity
Predicted
Rat
Host

Host Rabbit
Clonality Polyclonal
Isotype Rabbit IgG
Antigen Region 346-376

## **SPAK Antibody (Center) - Additional Information**

#### **Gene ID 27347**

#### **Other Names**

STE20/SPS1-related proline-alanine-rich protein kinase, Ste-20-related kinase, DCHT, Serine/threonine-protein kinase 39, STK39, SPAK

#### Target/Specificity

This SPAK antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 346-376 amino acids from the Central region of human SPAK.

#### **Dilution**

WB~~1:1000 IHC-P~~1:50~100

E~~Use at an assay dependent concentration.

### **Format**

Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is prepared by Saturated Ammonium Sulfate (SAS) precipitation followed by dialysis against PBS.

## **Storage**

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

#### **Precautions**

SPAK Antibody (Center) is for research use only and not for use in diagnostic or therapeutic procedures.

## **SPAK Antibody (Center) - Protein Information**

## Name STK39



Function Effector serine/threonine-protein kinase component of the WNK-SPAK/OSR1 kinase cascade, which is involved in various processes, such as ion transport, response to hypertonic stress and blood pressure (PubMed:16669787, PubMed:18270262, PubMed:21321328, PubMed:34289367). Specifically recognizes and binds proteins with a RFXV motif (PubMed:16669787, PubMed:21321328). Acts downstream of WNK kinases (WNK1, WNK2, WNK3 or WNK4): following activation by WNK kinases, catalyzes phosphorylation of ion cotransporters, such as SLC12A1/NKCC2, SLC12A2/NKCC1, SLC12A3/NCC, SLC12A5/KCC2 or SLC12A6/KCC3, regulating their activity (PubMed: 21321328). Mediates regulatory volume increase in response to hyperosmotic stress by catalyzing phosphorylation of ion cotransporters SLC12A1/NKCC2, SLC12A2/NKCC1 and SLC12A6/KCC3 downstream of WNK1 and WNK3 kinases (PubMed: 12740379, PubMed: 16669787, PubMed: 21321328). Phosphorylation of Na-K-Cl cotransporters SLC12A2/NKCC1 and SLC12A2/NKCC1 promote their activation and ion influx; simultaneously, phosphorylation of K-Cl cotransporters SLC12A5/KCC2 and SLC12A6/KCC3 inhibit their activity, blocking ion efflux (PubMed:16669787, PubMed:19665974, PubMed:21321328). Acts as a regulator of NaCl reabsorption in the distal nephron by mediating phosphorylation and activation of the thiazide-sensitive Na-Cl cotransporter SLC12A3/NCC in distal convoluted tubule cells of kidney downstream of WNK4 (PubMed: 18270262). Mediates the inhibition of SLC4A4, SLC26A6 as well as CFTR activities (By similarity). Phosphorylates RELT (By similarity).

#### **Cellular Location**

Cytoplasm. Nucleus. Note=Nucleus when caspase-cleaved.

#### **Tissue Location**

Predominantly expressed in brain and pancreas followed by heart, lung, kidney, skeletal muscle, liver, placenta and testis.

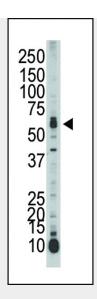
## **SPAK Antibody (Center) - Protocols**

Provided below are standard protocols that you may find useful for product applications.

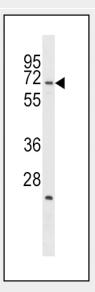
- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

# SPAK Antibody (Center) - Images



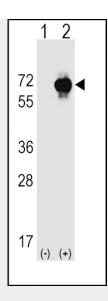


Western blot analysis of anti-SPAK Pab (Cat. #AP7968c) in mouse liver tissue lysate. SPAK (arrow) was detected using purified Pab. Secondary HRP-anti-rabbit was used for signal visualization with chemiluminescence.

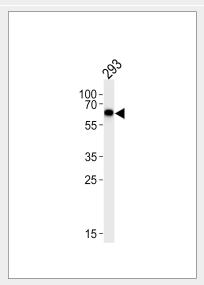


SPAK Antibody (A363) (Cat. #AP7968c) western blot analysis in U937 cell line lysates (35ug/lane). This demonstrates the SPAK antibody detected the SPAK protein (arrow).



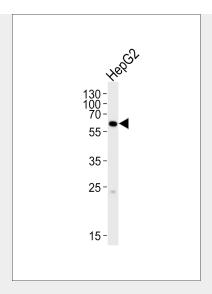


Western blot analysis of SPAK (arrow) using rabbit polyclonal SPAK Antibody (A363) (Cat. #AP7968c). 293 cell lysates (2 ug/lane) either nontransfected (Lane 1) or transiently transfected (Lane 2) with the SPAK gene.

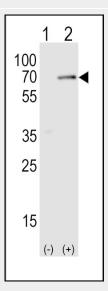


Western blot analysis of lysate from 293 cell line, using SPAK Antibody (A363)(Cat. #AP7968c). AP7968c was diluted at 1:1000 at each lane. A goat anti-rabbit  $IgG\ H\&L(HRP)$  at 1:5000 dilution was used as the secondary antibody. Lysate at 35ug.

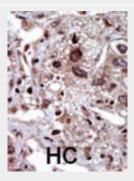




Western blot analysis of lysate from HepG2 cell line, using SPAK Antibody (A363)(Cat. #AP7968c). AP7968c was diluted at 1:1000. A goat anti-rabbit IgG H&L(HRP) at 1:10000 dilution was used as the secondary antibody. Lysate at 20ug.



Western blot analysis of SPAK (arrow) using rabbit polyclonal SPAK Antibody (A363) (Cat. #AP7968c). 293T cell lysates either nontransfected (Lane 1) or transiently transfected (Lane 2) with the SPAK gene.



Formalin-fixed and paraffin-embedded human cancer tissue reacted with the primary antibody, which was peroxidase-conjugated to the secondary antibody, followed by DAB staining. This data demonstrates the use of this antibody for immunohistochemistry; clinical relevance has not been evaluated. BC = breast carcinoma; HC = hepatocarcinoma.



# SPAK Antibody (Center) - Background

SPAK is a serine/threonine kinase containing an N-terminal series of proline and alanine repeats (PAPA box), followed by a serine/threonine kinase catalytic domain, a nuclear localization signal, a consensus caspase cleavage recognition motif, and a C-terminal region. Northern blot analysis detects ubiquitous expression, most abundantly in brain and pancreas.

SPAK can phosphorylate itself and an exogenous substrate in vitro. SPAK immunoprecipitates from transfected mammalian cells in a complex with another serine/threonine kinase that phosphorylates catalytically inactive SPAK. SPAK activates the p38 MAP kinase pathway in cotransfection assays. Full-length SPAK is expressed in the cytoplasm in transfected cells, while a mutant corresponding to caspase-cleaved STK39 localizes predominantly in the nucleus.

# **SPAK Antibody (Center) - References**

Dowd, B.F., et al., J. Biol. Chem. 278(30):27347-27353 (2003). Johnston, A.M., et al., Oncogene 19(37):4290-4297 (2000).

## **SPAK Antibody (Center) - Citations**

- STE20/SPS1-related proline/alanine-rich kinase is involved in plasticity of GABA signaling function in a mouse model of acquired epilepsy.
- From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene.
- PKCdelta acts upstream of SPAK in the activation of NKCC1 by hyperosmotic stress in human airway epithelial cells.
- Role for protein phosphatase 2A in the regulation of Calu-3 epithelial Na+-K+-2Cl-, type 1 co-transport function.