abcam

Product datasheet

Anti-PBK/SPK antibody [EPR21982] ab236871

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-PBK/SPK antibody [EPR21982]
Description	Rabbit monoclonal [EPR21982] to PBK/SPK
Host species	Rabbit
Specificity	IHC is recommended for human only.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 cell lysate; HEK293T, HeLa, HepG2, A431, U-87 MG, C6 and PC-12 whole cell lysates; Rat testis lysate. IHC-P: Human testis, gastric cancer and esophagus tissues. Flow Cyt (intra): HeLa and HepG2 cells. IP: HeLa whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR21982
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab236871 in the following tested applications.

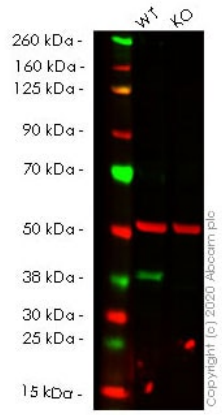
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50.
WB		1/1000. Detects a band of approximately 38 kDa (predicted molecular weight: 36 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC is recommended for human only.
IP		1/30.

Target

Function	Phosphorylates MAP kinase p38. Seems to be active only in mitosis. May also play a role in the activation of lymphoid cells. When phosphorylated, forms a complex with TP53, leading to TP53 destabilization and attenuation of G2/M checkpoint during doxorubicin-induced DNA damage.
Tissue specificity	Expressed in the testis and placenta. In the testis, restrictedly expressed in outer cell layer of seminiferous tubules.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. MAP kinase kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Phosphorylated; in a cell-cycle dependent manner at mitosis.

Images



Western blot - Anti-PBK/SPK antibody [EPR21982] (ab236871)

All lanes : Anti-PBK/SPK antibody [EPR21982] (ab236871) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PBK knockout HEK-293T cell lysate

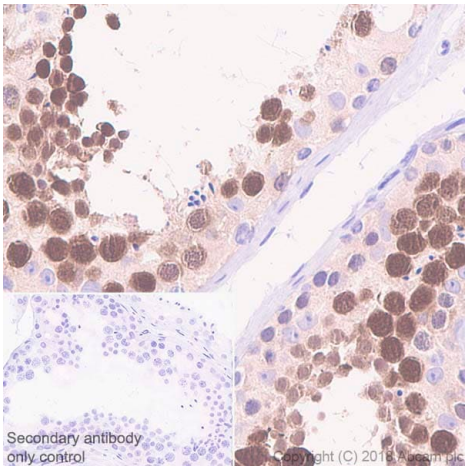
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 36 kDa
Observed band size: 40 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab236871 observed at 40 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab236871 was shown to react with PBK/SPK in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266827** (knockout cell lysate **ab257575**) was used. Wild-type HEK-293T and PBK knockout HEK-293T cell lysates were subjected to SDS-PAGE. ab236871 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

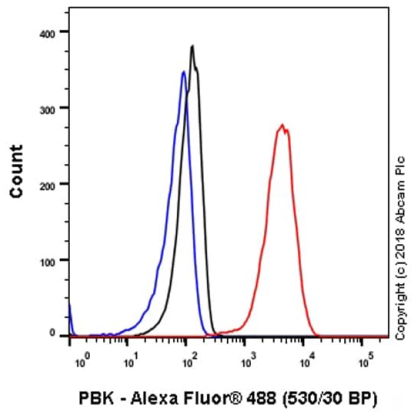


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PBK/SPK antibody [EPR21982] (ab236871)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling PBK/SPK with ab236871 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in germ cells of human testis (PMID:16982762; PMID:25909225) is observed. Counterstained with hematoxylin.

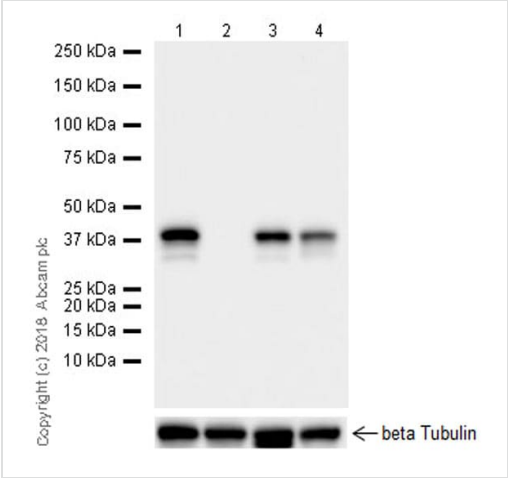
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Flow Cytometry (Intracellular) - Anti-PBK/SPK antibody [EPR21982] (ab236871)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling PBK/SPK with ab236871 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-PBK/SPK antibody [EPR21982] (ab236871)

All lanes : Anti-PBK/SPK antibody [EPR21982] (ab236871) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : PBK/SPK knockout HAP1 cell lysate
Lane 3 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 36 kDa
Observed band size: 38 kDa

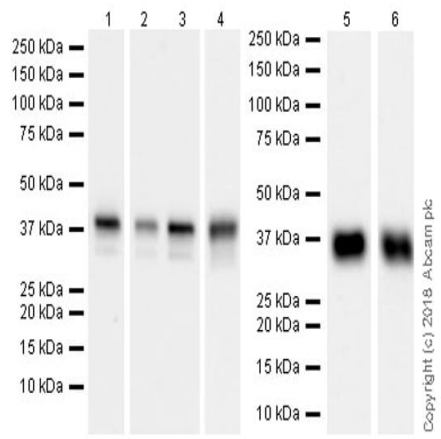
Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID:23547718; PMID:25909225).

ab236871 was shown to specifically react with PBK/SPK in wild-type HAP1 cells as signal was lost in PBK/SPK knockout cells. Wild-type and PBK/SPK knockout samples were subjected to SDS-PAGE. ab236871 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for

1 hour at room temperature before imaging.



Western blot - Anti-PBK/SPK antibody [EPR21982] (ab236871)

All lanes : Anti-PBK/SPK antibody [EPR21982] (ab236871) at 1/1000 dilution

Lane 1 : SW480 (human colorectal adenocarcinoma cell line) whole cell lysate at 20 µg
Lane 2 : A431 (human epidermoid carcinoma cell line) whole cell lysate at 20 µg
Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate at 20 µg
Lane 4 : Rat testis lysate at 20 µg
Lane 5 : C6 (rat glial tumor cell line) whole cell lysate at 10 µg
Lane 6 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate at 10 µg

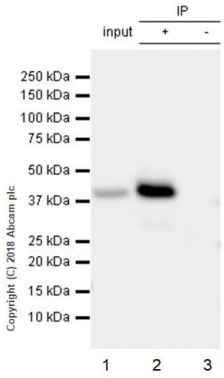
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 36 kDa
Observed band size: 38 kDa

Exposure time : Lanes 1-3: 26 seconds; Lane 4: 8 seconds; Lanes 5-6: 70 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID:23547718; PMID:25909225).



Immunoprecipitation - Anti-PBK/SPK antibody [EPR21982] (ab236871)

PBK/SPK was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab236871 at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab184276** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

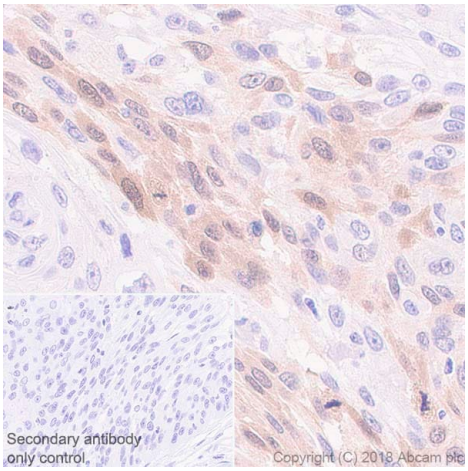
Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: **ab231871** IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab231871** in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

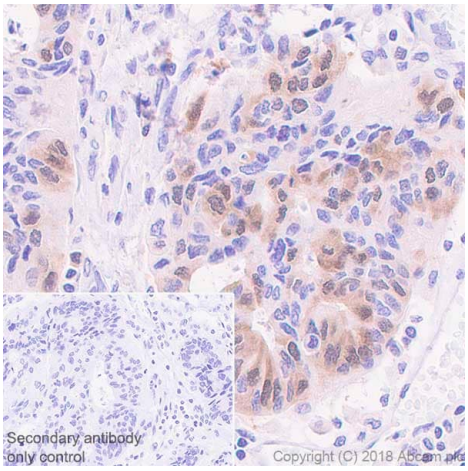


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PBK/SPK antibody [EPR21982] (ab236871)

Immunohistochemical analysis of paraffin-embedded human esophagus cancer tissue labeling PBK/SPK with ab236871 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in human esophagus cancer (PMID: 27919968) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

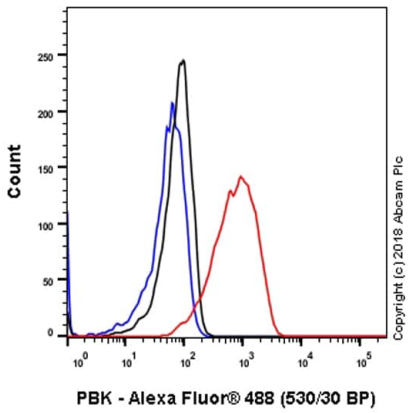


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PBK/SPK antibody [EPR21982] (ab236871)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling PBK/SPK with ab236871 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in human gastric cancer (PMID:26894977; PMID:27898655) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Flow Cytometry (Intracellular) - Anti-PBK/SPK antibody [EPR21982] (ab236871)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HepG2 (human liver hepatocellular carcinoma cell line) cell line labeling PBK/SPK with ab236871 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Anti-PBK/SPK antibody [EPR21982] (ab236871)

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