abcam

Product datasheet

Anti-PBK/SPK antibody [EPR21983] ab236872





2 References 10 Images

Overview

Product name Anti-PBK/SPK antibody [EPR21983]

Rabbit monoclonal [EPR21983] to PBK/SPK **Description**

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Human, rat and mouse testis tissue. Human gastric cancer tissue. WB: HeLa, HepG2,

> SW480, MCF7, A431, U-87 MG, Hepa1-6, C6, PC-12 and RAW 264.7 whole cell lysate. Human and rat testis lysate. Wild-type HAP1 cell lysate. IP: PBK/SPK IP in HepG2 whole cell lysate. Flow

Cyt (intra): HeLa and HepG2 cells.

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

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 EPR21983

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab236872 in the following tested applications.

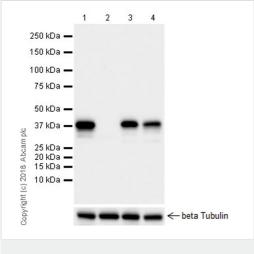
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 38 kDa (predicted molecular weight: 36 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
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 [EPR21983] (ab236872)

All lanes : Anti-PBK/SPK antibody [EPR21983] (ab236872) at 1/1000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: PBK/SPK knockout HAP1 cell lysate

Lane 3: HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 4 : HepG2 (human hepatocellular carcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

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

Exposure time: 15 seconds

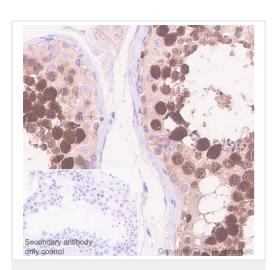
Blocking/Dilution buffer: 5% NFDM/TBST.

described in the literature (PMID:23547718; PMID:25909225).

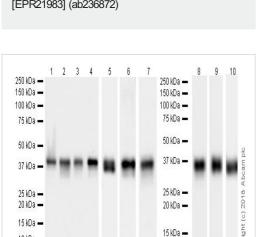
ab236872 was shown to specifically react with PBK/SPK in wildtype HAP1 cells as signal was lost in PBK/SPK knockout cells.

Wild-type and PBK/SPK knockout samples were subjected to
SDS-PAGE. ab236872 and <u>ab181602</u> (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 (<u>ab97051</u>) secondary antibody at 1/100,000 dilution for
1 hour at room temperature before imaging.

The molecular weight observed is consistent with what has been



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PBK/SPK antibody
[EPR21983] (ab236872)



Western blot - Anti-PBK/SPK antibody [EPR21983] (ab236872)

10 kDa -

10 kDa -

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling PBK/SPK with ab236872 at 1/4000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). 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 a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

All lanes : Anti-PBK/SPK antibody [EPR21983] (ab236872) at 1/1000 dilution

Lane 1: SW480 (human colorectal adenocarcinoma epithelial cell), whole cell lysate

Lane 2 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 3: A431 (human epidermoid carcinoma epithelial cell), whole cell lysate

Lane 4 : U-87 MG (human glioblastoma-astrocytoma epithelial cell), whole cell lysate

Lane 5: Hepa1-6 (mouse hepatoma epithelial cell), whole cell lysate

Lane 6: Human testis lysate

Lane 7: Rat testis lysate

Lane 8: C6 (rat glial tumor glial cell), whole cell lysate

Lane 9 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

Lane 10: RAW 264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage), whole cell lysate

Secondary

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

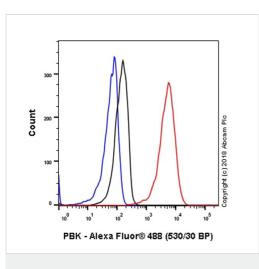
Predicted band size: 36 kDa **Observed band size:** 38 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-4 & 8-9: 15 seconds; Lanes 5 & 10: 3

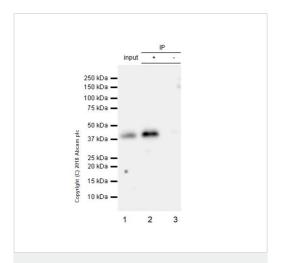
minutes; Lane 6: 70 seconds; Lane 7: 3 seconds.

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



Flow Cytometry (Intracellular) - Anti-PBK/SPK antibody [EPR21983] (ab236872)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling PBK/SPK with ab236872 at 1/500 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor[®] 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PBK/SPK antibody [EPR21983] (ab236872)

PBK/SPK was immunoprecipitated from 0.35 mg HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate with ab236872 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab236872 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

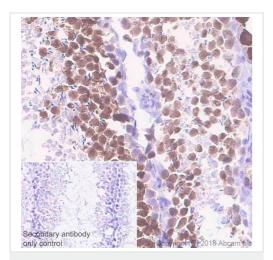
Lane 1: HepG2 whole cell lysate 10 µg (Input).

Lane 2: ab236872 IP in HepG2 whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab236872 in HepG2 whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

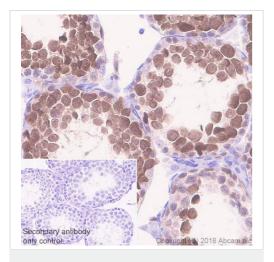
Exposure time: 10 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PBK/SPK antibody
[EPR21983] (ab236872)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling PBK/SPK with ab236872 at 1/4000 dilution, followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Positive staining in germ cells of rat testis (PMID:16982762; PMID:25909225) is observed. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

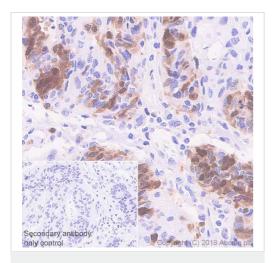
Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PBK/SPK antibody
[EPR21983] (ab236872)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling PBK/SPK with ab236872 at 1/4000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in germ cells of mouse testis (PMID:16982762; PMID:25909225) is observed. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PBK/SPK antibody
[EPR21983] (ab236872)

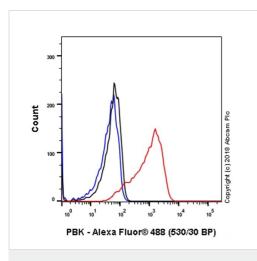
Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling PBK/SPK with ab236872 at 1/4000 dilution, followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Positive staining in human gastric cancer (PMID:26894977;

PMID:27898655) is observed. Counterstained with hematoxylin.

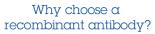
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



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Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cell line labeling PBK/SPK with ab236872 at 1/500 (red) compared with a Rabbit monoclonal lgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.





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Anti-PBK/SPK antibody [EPR21983] (ab236872)

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