abcam

Product datasheet

Anti-SIK3 antibody [EPR23554-71] ab255701

Recombinant RabMAb

8 Images

Overview

Product name Anti-SIK3 antibody [EPR23554-71]

Rabbit monoclonal [EPR23554-71] to SIK3 **Description**

Host species Rabbit

Specificity This antibody does not react with Mouse and Rat species for IHC application.

This antibody does not react with Human species for ICC and FC application.

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Whole cell lysates of: U-87 MG (human glioblastoma-astrocytoma epithelial cell), Neuro-2a

> (mouse neuroblastoma neuroblast), C6 (rat glial tumor glial cell). Tissue lysates from: human brain, mouse brain, rat brain. IHC-P: human ovarian cancer, human bladder cancer. ICC/IF: Neuro-2a (mouse neuroblastoma neuroblast) cells. Flow cyt. (Intra.): Neuro-2a (mouse

neuroblastoma neuroblast) 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 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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR23554-71

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab255701 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
ICC/IF		1/50.
IP		1/30.
WB		1/1000. Predicted molecular weight: 140 kDa.
IHC-P		1/100.
Flow Cyt (Intra)		1/50.

Target

Sequence similaritiesBelongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily.

Contains 1 protein kinase domain.

Contains 1 UBA domain.

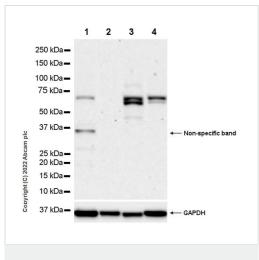
Post-translational

Phosphorylated at Thr-163 by STK11/LKB1 in complex with STE20-related adapter-alpha

modifications (STRADA) pseudo kinase and CAB39.

Cellular localization Cytoplasm. Locates to punctate structures within the cytoplasm on binding to YWHAZ.

Images



Western blot - Anti-SIK3 antibody [EPR23554-71] (ab255701)

All lanes : Anti-SIK3 antibody [EPR23554-71] (ab255701) at 1/1000 dilution

Lane 1: U-87 MG (human glioblastoma-astrocytoma epithelial cell), whole cell lysate

Lane 2: NIH:OVCAR-3 (human ovary adenocarcinoma epithelial cell), whole cell lysate

Lane 3 : Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate

Lane 4: C6 (rat glial tumor glial 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: 140 kDa **Observed band size:** 73,69 kDa

Exposure time: 3 minutes

Blocking / Dilution buffer and concentration: 5% NFDM/TBST

The expression profile/molecular weight observed is consistent with that described in the literature (PMID: 32542037).

Low expression: NIH:OVCAR-3 (PMID: PMID: 31762812)

2 250 kDa-250 kDa-150 kDa -150 kDa-100 kDa-100 kDa-75 kDa-75 kDa-50 kDa-37 kDa-37 kDa-25 kDa = 20 kDa = 25 kDa = 20 kDa = 15 kDa-15 kDa **–** 10 kDa-10 kDa

Western blot - Anti-SIK3 antibody [EPR23554-71] (ab255701)

All lanes : Anti-SIK3 antibody [EPR23554-71] (ab255701) at 1/1000 dilution

Lane 1: Human brain tissue lysate at 60 μg **Lane 2**: Mouse brain tissue lysate at 20 μg **Lane 3**: Rat brain tissue lysate at 20 μg

Secondary

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

Predicted band size: 140 kDa **Observed band size:** 73,69 kDa

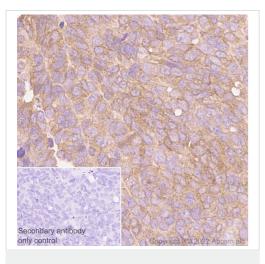
Blocking buffer and concentration: 5% NFDM/TBST

Exposure time:

Lane 1: 3 minutes

Lane 2 & 3: 26 seconds

The expression profile/molecular weight observed is consistent with that described in the literature (PMID: 32542037).

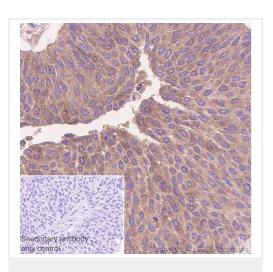


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIK3 antibody
[EPR23554-71] (ab255701)

Immunohistochemical analysis of paraffin-embedded Human ovarian cancer tissue labelling SIK3 with ab255701 at 1/100 (5.85 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining in human ovarian cancer (PMID: 21399663). The section was incubated with ab255701 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

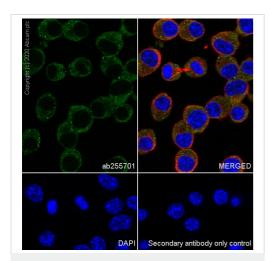


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIK3 antibody
[EPR23554-71] (ab255701)

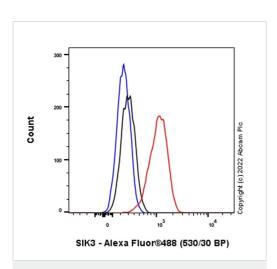
Immunohistochemical analysis of paraffin-embedded Human bladder cancer tissue labelling SIK3 with ab255701 at 1/100 (5.85 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining in human bladder cancer. The section was incubated with ab255701 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-SIK3 antibody [EPR23554-71] (ab255701)

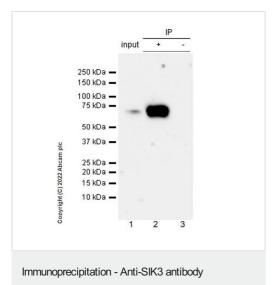


Flow Cytometry (Intracellular) - Anti-SIK3 antibody [EPR23554-71] (ab255701)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Neuro-2a (mouse neuroblastoma neuroblast) cells lebelling SIK3 with ab255701 at 1/50 (11.7 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed antibody at 1/1000 (2ug/mL) dilution (Green). Confocal image showing cytoplasmic staining in Neuro-2a cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2ug/mL) dilution.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling SIK3 with ab255701 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



[EPR23554-71] (ab255701)

SIK3 was immunoprecipitated from 0.35 mg Mouse brain tissue lysate 10 ug with ab255701 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab255701 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse brain tissue lysate 10 ug

Lane 2: ab255701 IP in Mouse brain tissue lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab255701 in Mouse brain tissue lysate

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

Exposure time: 41 seconds



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