abcam

Product datasheet

Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] ab185633

Recombinant RabMAb

11 References 11 Images

Overview

Product name Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671]

Description Rabbit monoclonal [EPR17671] to AKT1 + AKT2 + AKT3

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: AKT3 recombinant protein fragment (His-Tag®): aa351-479; AKT2 recombinant protein

fragment (His-Tag®): aa282-481; AKT1 recombinant protein fragment (His-Tag®): aa281-480; A549 whole cell lysate; Human fetal brain and fetal kidney lysates; Mouse brain lysate; Rat brain and heart lysates. IHC-P: Human cerebral cortex, Human adenocarcinoma of colon, mouse cerebral cortex and rat kidney tissues. ICC/IF: HeLa cells. Flow Cyt (intra): A549 cells. IP: A549

whole cell extract.

General notesThis 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

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Purity Protein A purified

Clonality Monoclonal
Clone number EPR17671

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab185633 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. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IP		1/50.
WB		1/2000. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
IHC-P		1/400. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

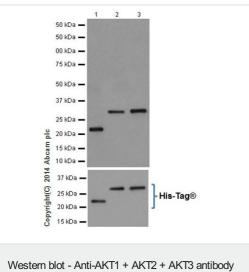
rarget		
Function	IGF-1 leads to the activation of AKT3, which may play a role in regulating cell survival. Capable of phosphorylating several known proteins. Truncated isoform 2/PKB gamma 1 without the second serine phosphorylation site could still be stimulated but to a lesser extent.	
Tissue specificity	In adult tissues, it is highly expressed in brain, lung and kidney, but weakly in heart, testis and liver. In fetal tissues, it is highly expressed in heart, liver and brain and not at all in kidney.	
Sequence similarities	Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. RAC subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 PH domain. Contains 1 protein kinase domain.	
Domain	Binding of the PH domain to the phosphatidylinositol 3-kinase alpha (PI(3)K) results in its targeting to the plasma membrane.	
Post-translational modifications	Phosphorylation on Thr-305 and Ser-472 is required for full activity (By similarity). Phosphorylated upon DNA damage, probably by ATM or ATR. Ubiquitinated. When fully phosphorylated and translocated into the nucleus, undergoes 'Lys-48'-polyubiquitination catalyzed by TTC3, leading to its degradation by the proteasome.	

Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation.

Images

Cellular localization

Target



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) **All lanes :** Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) at 1/5000 dilution

Lane 1: AKT3 recombinant protein fragment (His-Tag®): aa351-479

Lane 2: AKT2 recombinant protein fragment (His-Tag®): aa282-481

Lane 3 : AKT1 recombinant protein fragment (His-Tag®): aa281-480

Lysates/proteins at 0.01 µg per lane.

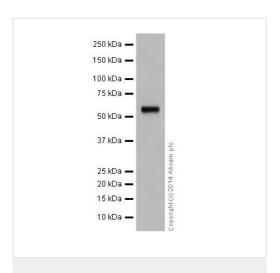
Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 56 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

The bottom panel is probed with an anti-His tag antibody.



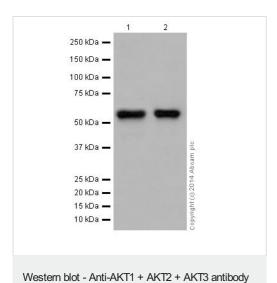
Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) at 1/2000 dilution + A549 (Human lung carcinoma) whole cell lysates at 20 μg

Secondary

Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 56 kDa Observed band size: 56 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



[EPR17671] (ab185633)

All lanes : Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) at 1/2000 dilution

Lane 1 : Human fetal brain lysates

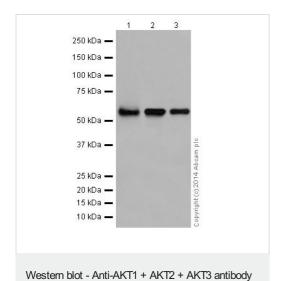
Lane 2 : Human fetal kidney lysates

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 56 kDa Observed band size: 56 kDa



Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633) at 1/2000 dilution

Lane 1 : Mouse brain lysates
Lane 2 : Rat brain lysates
Lane 3 : Rat heart lysates

Lysates/proteins at 10 µg per lane.

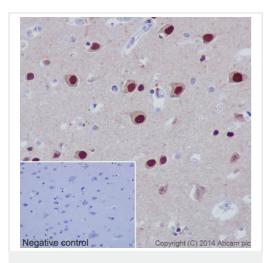
Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 56 kDa **Observed band size:** 56 kDa

[EPR17671] (ab185633)

Blocking/Dilution buffer: 5% NFDM/TBST.

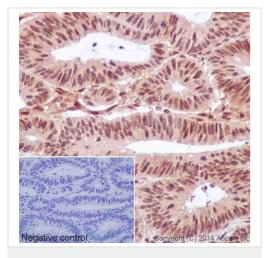


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

Immunohistochemical analysis of paraffin-embedded
Human cerebral cortex tissue labeling AKT1 + AKT2 + AKT3 with
ab185633 at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L
(HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and
weak cytoplasmic staining on neurons of the Human cerebral
cortex is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

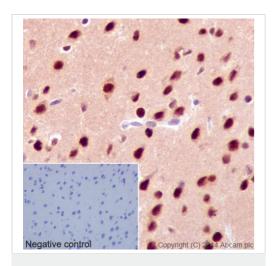


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

Immunohistochemical analysis of paraffin-embedded
Human adenocarcinoma of colon tissue labeling AKT1 + AKT2 +
AKT3 with ab185633 at 1/400 dilution, followed by Goat Anti-Rabbit
IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.
Nuclear and weak cytoplasmic staining on Human adenocarcinoma
of colon is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

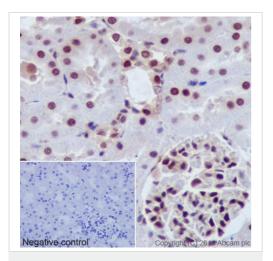


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

Immunohistochemical analysis of paraffin-embedded Mouse cerebral cortex tissue labeling AKT1 + AKT2 + AKT3 with ab185633 at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and weak cytoplasmic staining on neurons of the mouse cerebral cortex is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

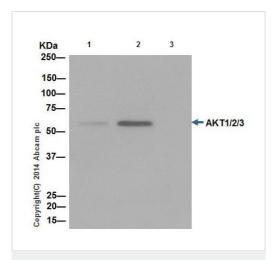


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling AKT1 + AKT2 + AKT3 with ab185633 at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and weak cytoplasmic staining on rat kidney is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

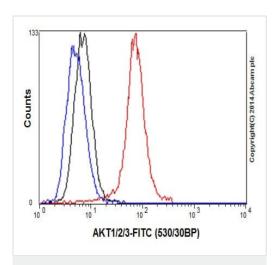
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

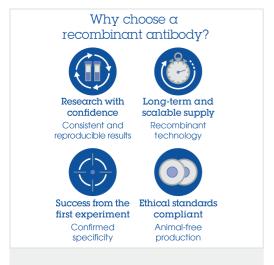
AKT1 + AKT2 + AKT3 was immunoprecipitated from 1mg of A549 (Human lung carcinoma) whole cell extract with ab185633 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab185633 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: A549 whole cell extract 10 μ g (Input). Lane 2: ab185633 IP in A549 whole cell extract. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab185633 in A549 whole cell extract. Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixedA549 (Human lung carcinoma) cells labeling AKT1 + AKT2 + AKT3 with ab185633 at 1/50 dilution (red) compared with a rabbit monoclonal lgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (FITC) at 1/150 dilution was used as the secondary antibody.



Anti-AKT1 + AKT2 + AKT3 antibody [EPR17671] (ab185633)

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