abcam

Product datasheet

Anti-AKT1 + AKT2 antibody [EPR17062] ab182729

Recombinant RabMAb

16 References 14 Images

Overview

Product name	Anti-AKT1 + AKT2 antibody [EPR17062]
Description	Rabbit monoclonal [EPR17062] to AKT1 + AKT2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human AKT1 fragment recombinant protein; Human AKT2 fragment recombinant protein; Human fetal heart and fetal kidney lysates; HeLa, MCF7, C6, RAW 264.7 and NIH/3T3 whole cell lysates; Mouse and rat brain and heart lysates. IHC-P: Human tonsil, Human bladder cancer, mouse stomach and rat cerebrum tissues. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa and NIH/3T3 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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR17062
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab182729 in the following tested applications.

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

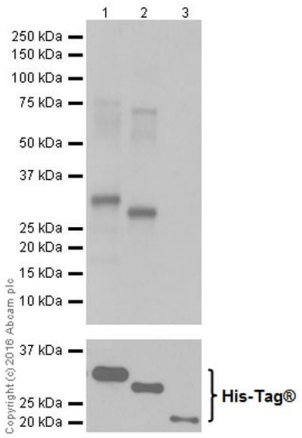
Application	Abreviews	Notes
Flow Cyt (Intra)		1/600.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/5000. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
ICC/IF		1/500.
IP		1/40.

Target

Relevance The serine/threonine kinase AKT (protein kinase B or PKB) has a central role in the regulation of several signaling pathways controlling cell proliferation, apoptosis, angiogenesis, and diabetes. In humans, there are three genes in the "AKT family": AKT1, AKT2, and AKT3. AKT1 is catalytically inactive in serum starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet derived growth factor. The activation is rapid and specific. In the developing nervous system AKT is a critical mediator of growth factor induced neuronal survival. Survival factors can suppress apoptosis in a transcription independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. AKT2 is a putative oncogene and is a general protein kinase capable of phophorylating several known proteins. AKT2 is amplified and overexpressed in some human carcinomas. AKT2 acts primarily as a regulator of glucose metabolism.

Cellular localization ATK1: Cytoplasm. Nucleus. Cell membrane. Note: Nucleus after activation by integrin-linked protein kinase 1 (ILK1). Nuclear translocation is enhanced by interaction with TCL1A.

Images



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

All lanes : Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729) at 1/1000 dilution

Lane 1 : Human AKT1 fragment recombinant protein
Lane 2 : Human AKT2 fragment recombinant protein
Lane 3 : Human AKT3 fragment recombinant protein

Lysates/proteins at 0.02 µg per lane.

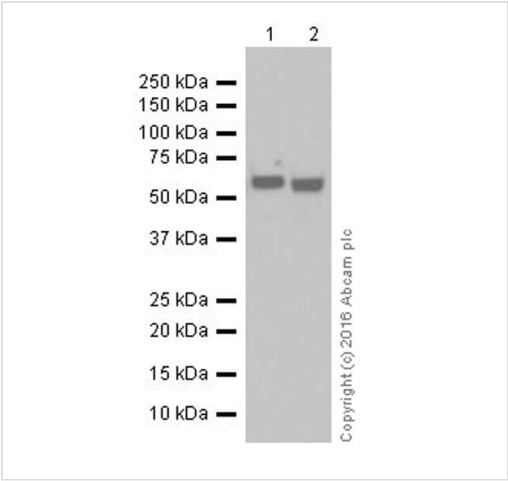
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 56 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Human AKT1 fragment recombinant protein contains aa281-480 with a His-Tag®. Human AKT2 fragment recombinant protein contains aa282-481 with a His-Tag®. Human AKT3 fragment recombinant protein contains aa351-479 with a His-Tag®.



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

All lanes : Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729) at 1/5000 dilution

Lane 1 : Human fetal heart lysate
Lane 2 : Human fetal kidney lysate

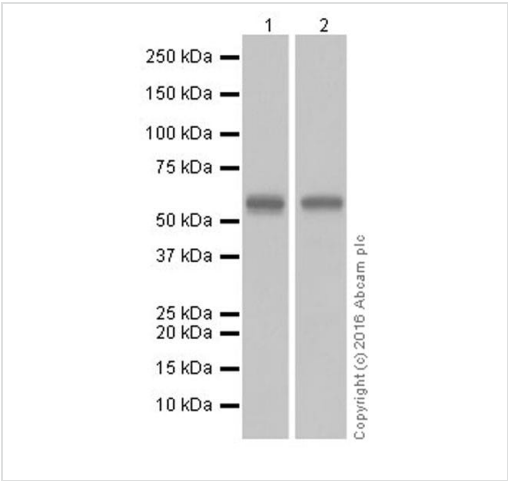
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

All lanes : Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

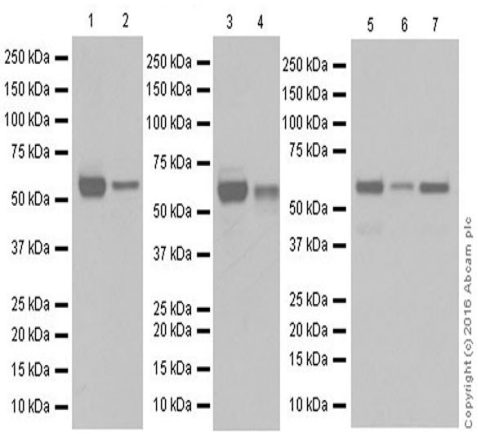
Lysates/proteins at 20 µg per lane.

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

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 8 seconds; Lane 2: 2 seconds.



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

All lanes : Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729) at 1/5000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Rat brain lysate
Lane 4 : Rat heart lysate
Lane 5 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 6 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 7 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

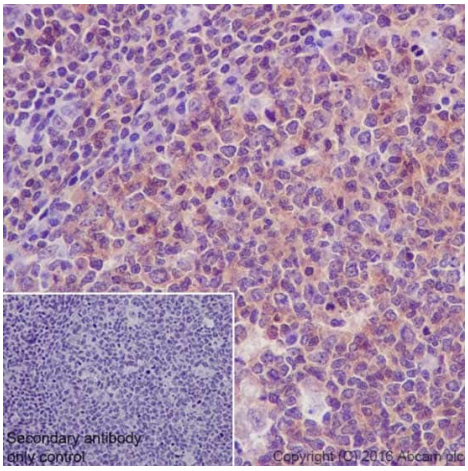
Lysates/proteins at 10 µg per lane.

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

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 2,3 and 4: 5 seconds; Lane 5,6 and 7: 3 seconds.

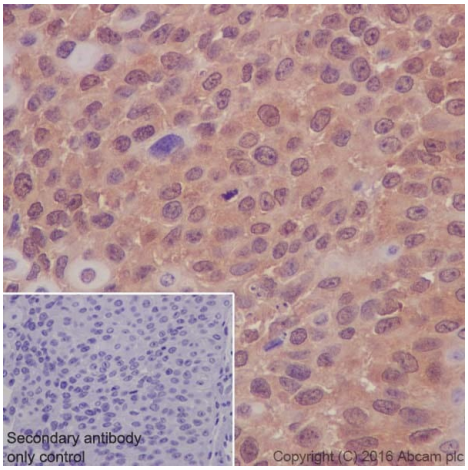


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling AKT1 + AKT2 with ab182729 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm and nuclear staining on Human tonsil is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

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

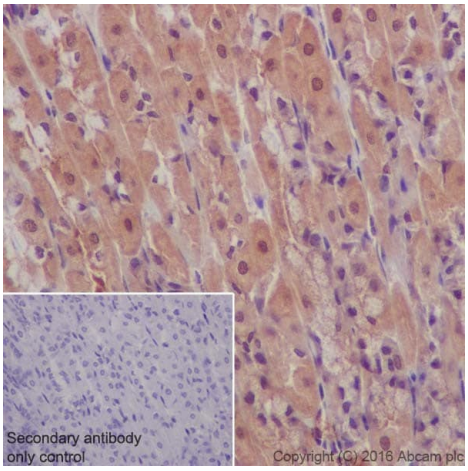


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Immunohistochemical analysis of paraffin-embedded Human bladder cancer tissue labeling AKT1 + AKT2 with ab182729 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm and nuclear staining on Human bladder cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

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

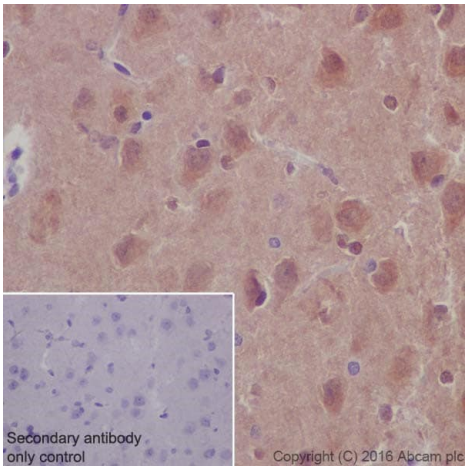


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Immunohistochemical analysis of paraffin-embedded Mouse stomach tissue labeling AKT1 + AKT2 with ab182729 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm and nuclear staining on Mouse stomach is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

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

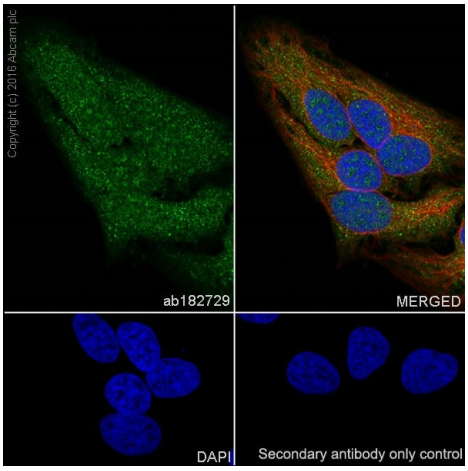


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling AKT1 + AKT2 with ab182729 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm and nuclear staining on Rat cerebrum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

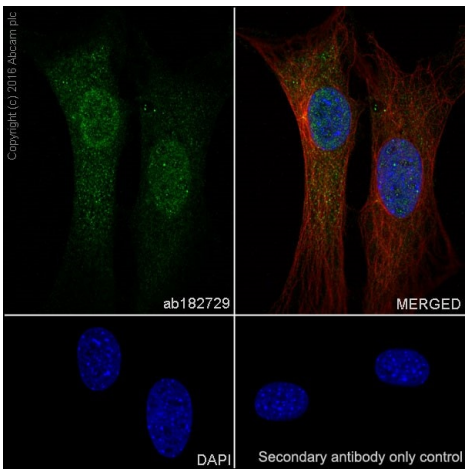
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling AKT1 + AKT2 with ab182729 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasmic staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

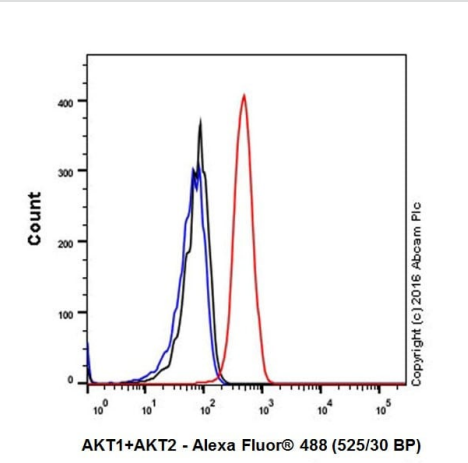
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

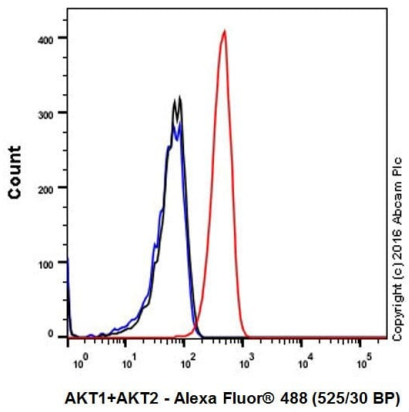
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling AKT1 + AKT2 with ab182729 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasmic staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** at 1/1000 dilution.



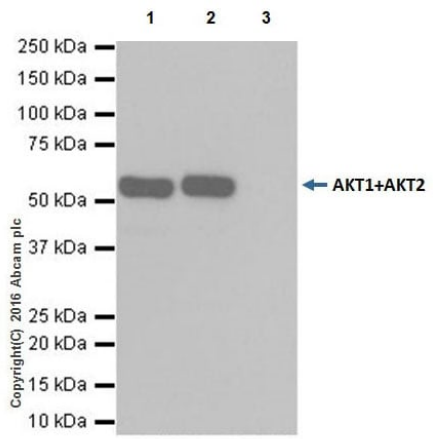
Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling AKT1 + AKT2 with ab182729 at 1/600 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A]-Isotype control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling AKT1 + AKT2 with ab182729 at 1/600 dilution (red) compared with a RabbitIgG,monoclonal [EPR25A]-Isotype control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

AKT1/2 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab182729 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab182729 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

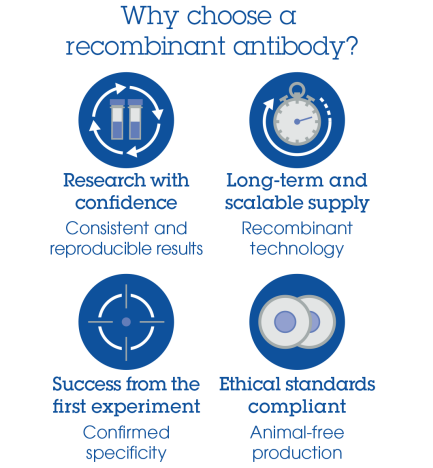
Lane 1: HeLa whole cell lysate, 10µg (Input).

Lane 2: ab182729 IP in HeLa whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A]-Isotype Control (**ab172730**) instead of ab182729 in HeLa whole cell lysate.

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

Exposure time: 30 seconds.



Anti-AKT1 + AKT2 antibody [EPR17062] (ab182729)

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