abcam

Product datasheet

Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free ab250634

Recombinant RabMAb

14 Images

Overview

Product name Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free

Description Rabbit monoclonal [EPR17062] to AKT1 + AKT2 - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human, Recombinant fragment

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

General notes ab250634 is the carrier-free version of ab182729.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR17062

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab250634 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
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
ICC/IF		Use at an assay dependent concentration.

Target

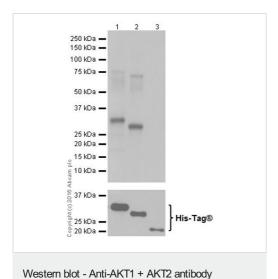
Relevance

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



[EPR17062] - BSA and Azide free (ab250634)

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 $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

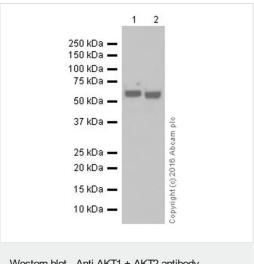
Predicted band size: 56 kDa

Exposure time: 1 second

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

Blocking and 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] - BSA and Azide free (ab250634) **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

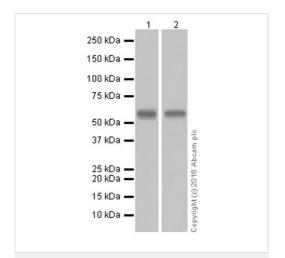
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 30 seconds

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634) **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

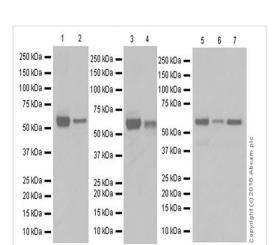
Lysates/proteins at 20 µg per lane.

Secondary

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

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

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.



Western blot - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634) Blocking and dilution buffer: 5% NFDM/TBST.

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

All lanes : Anti-AKT1 + AKT2 antibody [EPR17062] (<u>ab182729</u>) 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

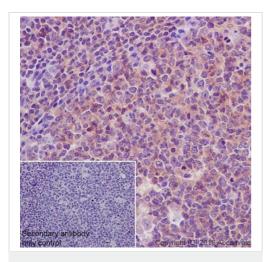
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

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

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: Lanes 1-4: 5 seconds; Lanes 5-7: 3 seconds.

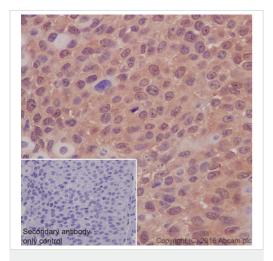


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 antibody

[EPR17062] - BSA and Azide free (ab250634)

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

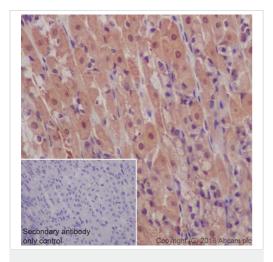
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling AKT1 + AKT2 with <u>ab182729</u> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Cytoplasm and nuclear staining on Human tonsil is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <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 antibody
[EPR17062] - BSA and Azide free (ab250634)

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human bladder cancer tissue labeling AKT1 + AKT2 with <u>ab182729</u> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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 <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.

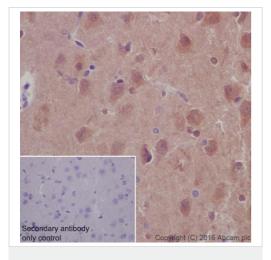


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 antibody

[EPR17062] - BSA and Azide free (ab250634)

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

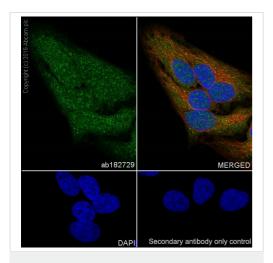
Immunohistochemical analysis of paraffin-embedded Mouse stomach tissue labeling AKT1 + AKT2 with <u>ab182729</u> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Cytoplasm and nuclear staining on Mouse stomach is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <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 antibody
[EPR17062] - BSA and Azide free (ab250634)

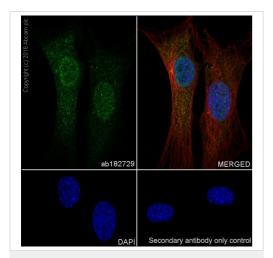
This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling AKT1 + AKT2 with <u>ab182729</u> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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 <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.



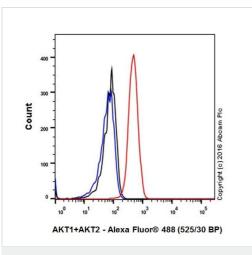
Immunocytochemistry/ Immunofluorescence - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634)

This data was developed using **ab182729**, the same antibody clone in a different buffer formulation.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.

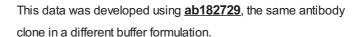


Immunocytochemistry/ Immunofluorescence - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634)

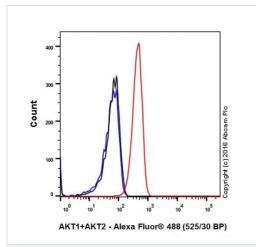
This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.lmmunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling AKT1 + AKT2 with <u>ab182729</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (<u>ab150077</u>) 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 <u>ab195889</u> (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 <u>ab150077</u> at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634)



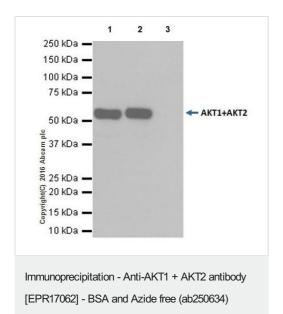
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling AKT1 + AKT2 with <u>ab182729</u> at 1/600 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A]-Isotype control (<u>ab172730</u>) (black) and an unlabelled 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.



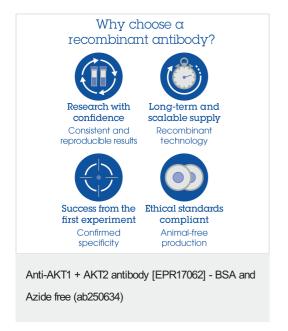
Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 antibody [EPR17062] - BSA and Azide free (ab250634)

This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.

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 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.



This data was developed using <u>ab182729</u>, the same antibody clone in a different buffer formulation.AKT1/2 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with <u>ab182729</u> at 1/40 dilution. Western blot was performed from the immunoprecipitate using <u>ab182729</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution. Lane 1: HeLa whole cell lysate, 10µg (Input). Lane 2: <u>ab182729</u> IP in HeLa whole cell lysate. Lane 3: Rabbit IgG,monoclonal [EPR25A]-Isotype Control (<u>ab172730</u>) instead of <u>ab182729</u> in HeLa whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- · Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors