# abcam

# Product datasheet

# Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] ab179463

יובעדער RabMAb

#### 275 References 画像数 12

#### 製品の概要

製品名 Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798]

製品の詳細 Rabbit monoclonal [EPR16798] to AKT1 + AKT2 + AKT3

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human, Xenopus laevis, Xenopus tropicalis

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

ポジティブ・コントロール WB: MCF7, HeLa, Hep G2 and A549 whole cell lysates; Human fetal brain, heart and kidney

> lysates; Mouse and Rat brain, heart, kidney and spleen lysates; Xenopus muscle lysate; AKT2 and AKT3 recombinant proteins. IHC-P: Human kidney, Mouse and Rat cerebral cortex. ICC/IF:

K562 cells. Flow: A549 cells. IP: MCF7 whole cell lysate

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 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.

バッファー Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR16798

### アプリケーション

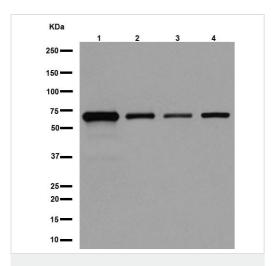
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アプリケーション	Abreviews	特記事項
WB		1/10000. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/100.
Flow Cyt (Intra)		1/330.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

## ターゲット情報

機能	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.
組織特異性	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.
配列類似性	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.
ドメイン	Binding of the PH domain to the phosphatidylinositol 3-kinase alpha (Pl(3)K) results in its targeting to the plasma membrane.
翻訳後修飾	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.
細胞内局在	Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation.

#### 画像



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

**All lanes :** Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463) at 1/10000 dilution

**Lane 1 :** MCF7 (Human breast adenocarcinoma cell line) whole cell lysates

**Lane 2**: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates

**Lane 3**: Hep G2 (Human liver hepatocellular carcinoma) whole cell lysates

Lane 4: A549 (Human lung carcinoma) whole cell lysates

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** 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.

ab179463 MERGED

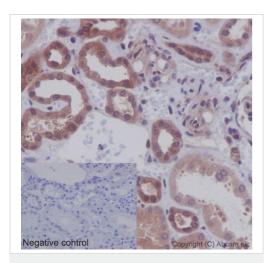
DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling AKT1 + AKT2 + AKT3 with ab179463 at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/400 dilution (green). Cytoplasm and nuclear staining on K562 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows;

ab179463 at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
 <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.

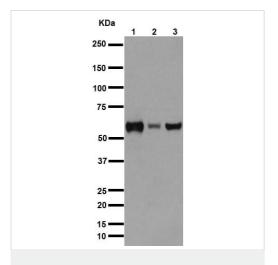


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling AKT1 + AKT2 + AKT3 with ab179463 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse lgG. Cytoplasm and nucleus staining on Human renal cortex is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

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



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

**All lanes :** Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463) at 1/1000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate

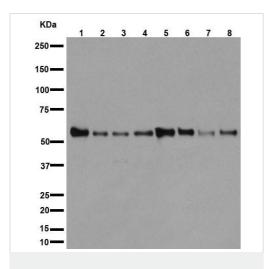
Lysates/proteins at 10 µg per lane.

#### **Secondary**

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

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

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

**All lanes :** Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463) at 1/1000 dilution

Lane 1 : Mouse brain lysate

Lane 2: Mouse heart lysate

Lane 3: Mouse kidney lysate

Lane 4: Mouse spleen lysate

Lane 5: Rat brain lysate

Lane 6: Rat heart lysate

Lane 7: Rat kidney lysate

Lane 8: Rat spleen lysate

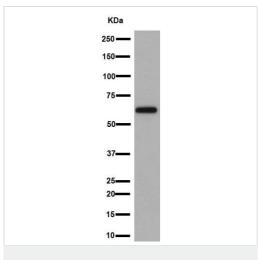
Lysates/proteins at 10 µg per lane.

#### Secondary

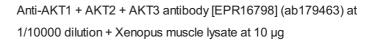
**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

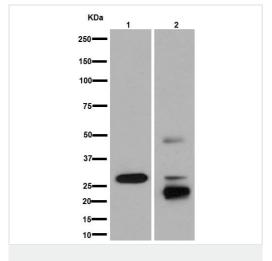


#### Secondary

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.



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

**All lanes :** Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463) at 1/10000 dilution

Lane 1 : AKT2 recombinant protein (HIS-tag): aa282-481

Lane 2 : AKT3 recombinant protein (HIS-tag) :aa351-479

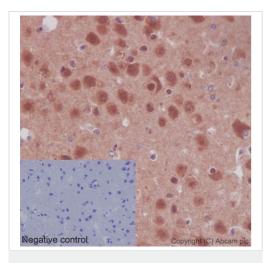
Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 56 kDa **Observed band size:** 18,26 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



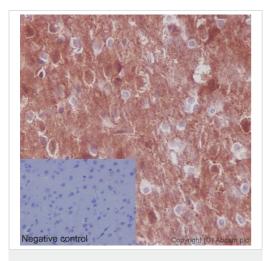
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

Immunohistochemical analysis of paraffin-embedded

Mouse cerebral cortex tissue labeling AKT1 + AKT2 + AKT3 with
ab179463 at 1/250 dilution, followed by prediluted HRP Polymer for
Rabbit/Mouse IgG. Cytoplasm and nucleus staining on Mouse
cerebral cortex is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

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

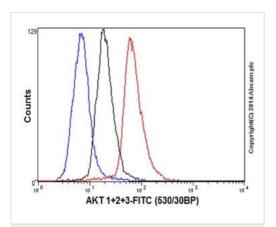


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

Immunohistochemical analysis of paraffin-embedded Rat cerebral cortex tissue labeling AKT1 + AKT2 + AKT3 with ab179463 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasm and nucleus staining on Rat cerebral cortex is observed. Counter stained with Hematoxylin.

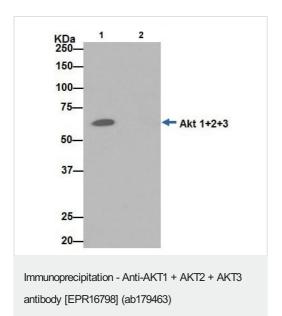
Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

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

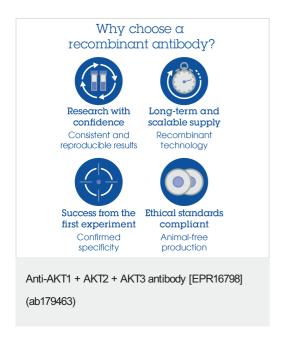


Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (ab179463)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed A549 (Human lung carcinoma) cells labeling AKT1 + AKT2 + AKT3 with ab179463 at 1/330 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



AKT1 + AKT2 + AKT3 was immunoprecipitated from 1mg of MCF7 (Human breast adenocarcinoma cell line) whole cell extract with ab179463 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab179463 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: MCF7 whole cell extract. Lane 2: PBS instead of MCF7 whole cell extract. Blocking and dilution buffer and concentration: 5% NFDM/TBST.



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