abcam

Product datasheet

Anti-AKT1 (phospho S473) antibody [EP2109Y] ab81283

Recombinant RabMAb

6 Abreviews 85 References 13 Images

Overview

Product name	Anti-AKT1 (phospho S473) antibody [EP2109Y]
Description	Rabbit monoclonal [EP2109Y] to AKT1 (phospho S473)
Specificity	ab81283 detects AKT1 phosphorylated at Serine 473. The region of AKT1 surrounding S473 has a high degree of similarity to the corresponding regions in AKT2 and AKT3 and thus may cross react with these proteins if phosphorylated on the corresponding serine residue.
Tested applications	**Suitable for:** ICC/IF, IHC-Fr, IHC-P, WB **Unsuitable for:** Flow Cyt or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	A phospho specific peptide corresponding to residues surrounding Ser473 of human AKT1.
Positive control	3T3 cell lysate treated with PDGF. Cervical carcinoma.
General notes	This product is a recombinant rabbit monoclonal antibody. Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487. A trial size is available to purchase for this antibody. Alternative versions available: Anti-AKT1 (phospho S473) antibody (Alexa Fluor® 488) [EP2109Y] (ab194198) Anti-AKT1 (phospho S473) antibody (Alexa Fluor® 647) [EP2109Y] (ab194200) Anti-AKT1 (phospho S473) antibody (HRP) [EP2109Y] (ab194201)

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. Avoid freeze / thaw cycle.
Storage buffer	PBS 49%,Sodium azide 0.01%,Glycerol 50%,BSA 0.05%
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EP2109Y

Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab81283** 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	★★★★★	1/100 - 1/250.
IHC-Fr	★★★★★	Use at an assay dependent concentration.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★☆	1/5000 - 1/10000. Predicted molecular weight: 56 kDa.Can be blocked with AKT1 peptide (ab171724) or AKT1 peptide (ab217601). Abcam recommends using BSA as the blocking agent.

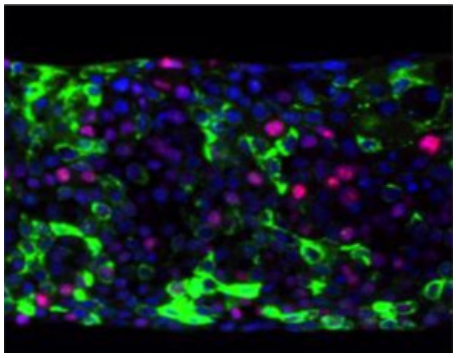
Application notes	Is unsuitable for Flow Cyt or IP.

Target

Function	Plays a role as a key modulator of the AKT-mTOR signaling pathway controlling the tempo of the process of newborn neurons integration during adult neurogenesis, including correct neuron positioning, dendritic development and synapse formation (By similarity). General protein kinase capable of phosphorylating several known proteins. Phosphorylates TBC1D4. Signals downstream of phosphatidylinositol 3-kinase (PI(3)K) to mediate the effects of various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin and insulin-like growth factor I (IGF-I). Plays a role in glucose transport by mediating insulin-induced translocation of the GLUT4 glucose transporter to the cell surface. Mediates the antiapoptotic effects of IGF-I. Mediates insulin-stimulated protein synthesis by phosphorylating TSC2 at 'Ser-939' and 'Thr-1462', thereby activating mTORC1 signaling and leading to both phosphorylation of 4E-BP1 and in activation of RPS6KB1. Promotes glycogen synthesis by mediating the insulin-induced activation of glycogen synthase. The activated form can suppress FoxO gene transcription and promote cell cycle progression. Essential for the SPATA13-mediated regulation of cell migration and adhesion assembly and disassembly.
Tissue specificity	Expressed in all human cell types so far analyzed. The Tyr-176 phosphorylated form shows a significant increase in expression in breast cancers during the progressive stages i.e. normal to hyperplasia (ADH), ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC) and lymph node metastatic (LNMM) stages.
Involvement in disease	Defects in AKT1 are a cause of susceptibility to breast cancer (BC) [MIM:114480]. A common malignancy originating from breast epithelial tissue. Breast neoplasms can be distinguished by their histologic pattern. Invasive ductal carcinoma is by far the most common type. Breast cancer is etiologically and genetically heterogeneous. Important genetic factors have been indicated by familial occurrence and bilateral involvement. Mutations at more than one locus can be involved in different families or even in the same case. Defects in AKT1 are associated with colorectal cancer (CRC) [MIM:114500]. Defects in AKT1 are associated with susceptibility to ovarian cancer [MIM:604370]; also called susceptibility to familial breast-ovarian cancer type 1 (BROVCA1).
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. The PH domain mediates interaction with TNK2 and Tyr-176 is also essential for this interaction. The AGC-kinase C-terminal mediates interaction with THEM4.
Post-translational modifications	Phosphorylation on Thr-308, Ser-473 and Tyr-474 is required for full activity. Activated TNK2 phosphorylates it on Tyr-176 resulting in its binding to the anionic plasma membrane phospholipid PA. This phosphorylated form localizes to the cell membrane, where it is targeted by PDPK1 and PDPK2 for further phosphorylations on Thr-308 and Ser-473 leading to its activation. Ser-473 phosphorylation by mTORC2 favors Thr-308 phosphorylation by PDPK1. Ser-473 phosphorylation is enhanced by interaction with AGAP2 isoform 2 (PIKE-A). Ser-473 phosphorylation is enhanced in focal cortical dysplasias with Taylor-type balloon cells. Ubiquitinated; undergoes both 'Lys-48'- and 'Lys-63'-linked polyubiquitination. TRAF6-induced 'Lys-63'-linked AKT1 ubiquitination is critical for phosphorylation and activation. When ubiquitinated, it translocates to the plasma membrane, where it becomes phosphorylated. When fully phosphorylated and translocated into the nucleus, undergoes 'Lys-48'-polyubiquitination catalyzed by TTC3, leading to its degradation by the proteasome.
Cellular localization	Cytoplasm. Nucleus. Cell membrane. Nucleus after activation by integrin-linked protein kinase 1 (ILK1). Nuclear translocation is enhanced by interaction with TCL1A. Phosphorylation on Tyr-176 by TNK2 results in its localization to the cell membrane where it is targeted for further phosphorylations on Thr-308 and Ser-473 leading to its activation and the activated form translocates to the nucleus.

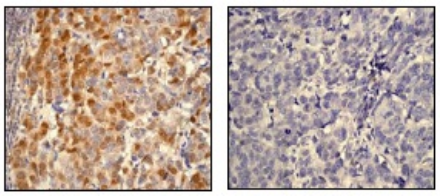
Anti-AKT1 (phospho S473) antibody [EP2109Y] images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

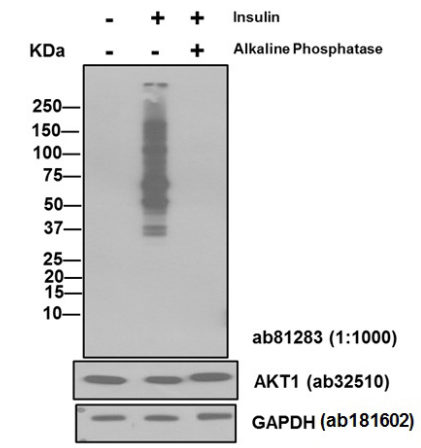
Image from Henken FE et al., Mol Cancer. 2011 Jun 10;10:71. Fig 7.; doi:10.1186/1476-4598-10-71; 10 June 2011, Molecular Cancer 2011, 10:71

Immunohistochemical analysis of Human HPV16 immortalized keratinocytes transfected with non-targeting siRNA, staining AKT1 (phospho S473) (green) with ab81283. Antigen retrieval was performed by heat mediation in citrate buffer (pH 6). Samples were blocked with 10% goat serum before incubating with primary antibody (1/100). Fluoroscein-conjugated tyramide was used to detect staining.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

ab81283, at 1/100 dilution, staining AKT1 in untreated (left panel) and Phosphatase-treated (right panel) cervical carcinoma by Immunohistochemistry using formalin-fixed, paraffin-embedded tissue



Western blot - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

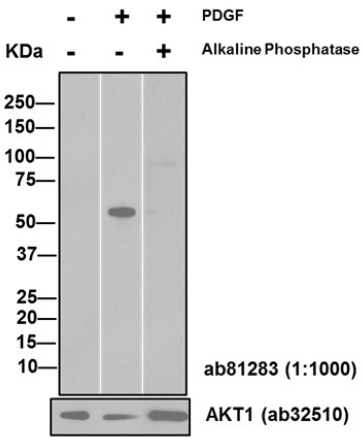
All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 1/1000 dilution

Lane 1 : HEK293 cell lysate
Lane 2 : HEK293 cell lysate
Lane 3 : HEK293 cell lysate

Predicted band size : 56 kDa

Insulin treatment: cells were starved overnight and then treated for 20 min (Insulin) at 100 ng/ml.

Phosphatase treatment: membrane strips were incubated with 200 ul of phosphatase (150 U/ml) at 37 degrees for 1 hour.



Western blot - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

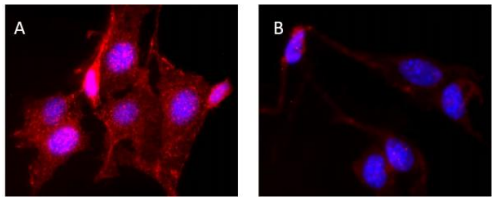
All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 1/1000 dilution

Lane 1 : NIH/3T3 cell lysate
Lane 2 : NIH/3T3 cell lysate
Lane 3 : NIH/3T3 cell lysate

Predicted band size : 56 kDa

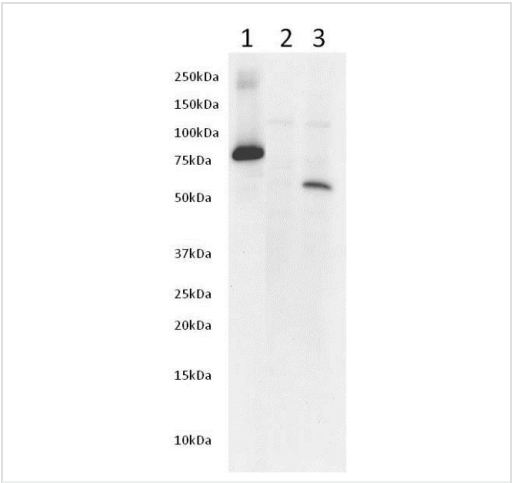
PDGF treatment: cells were starved overnight and then treated for 1 h with PDGF at 100 ng/ml.

Phosphatase treatment: membrane strips were incubated with 200 ul of phosphatase (150 U/ml) at 37 degrees for 1 hour.



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (phospho S473) [EP2109Y] antibody (ab81283)

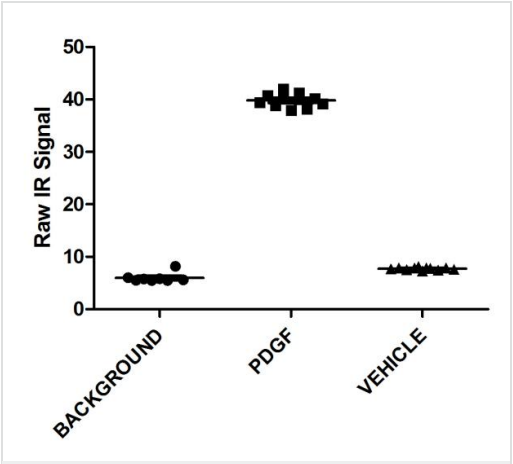
NIH3T3 cells starved overnight and treated with PDGF 50ng/mL for 1 hour (A) or vehicle (B). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton-X100. Primary antibody ab81283 was used at 1:4000 dilution and secondary antibody Dylight GAR594 (ab96897) at 1:1000 dilution.



Western blot - Anti-AKT1 (phospho S473) [EP2109Y] antibody (ab81283)

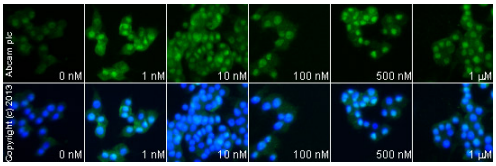
Predicted band size : 56 kDa

Primary : All Lanes : Anti AKT1 (phospho S473) antibody (ab81283) at 1:5000 dilution. Lane 1 = AKT1 (His tag) full length recombinant protein ab62279 - 50ng. Lane 2 = NIH3T3 serum starved overnight ? 15ug. Lane 3 = NIH3T3 serum starved overnight and treated with PDGF-AB 50ng/mL for 1 hour ? 15ug. Secondary : Lanes 1-3 : Goat polyclonal to Rabbit IgG ? H&L ? Pre-Adsorbed (HRP) at 1:5000 developed using the ECL technique. Performed under reducing conditions (50mM DTT ? Sample heated at 60?C). Predicted band size : 56kDa. Observed band size : 56kDa. Blocking step: 5% Milk in 50mM Tris+0.05% Tween for 1 hour at RT. Primary antibody buffer: 5% BSA in 50mM Tris+0.05% Tween overnight. Secondary antibody buffer: 5% Milk in 50mM Tris+0.05% Tween for 2 hours at RT. Exposure time : 5 minutes



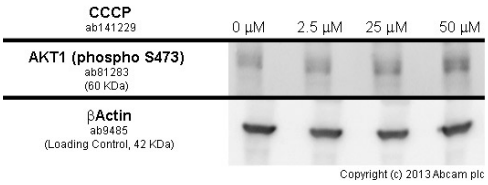
In-Cell ELISA - Anti-AKT1 (phospho S473) [EP2109Y] antibody (ab81283)

NIH3T3 cells were starved overnight and treated with PDGF 50ng/mL or vehicle control for 1 hour prior to fixation with 4% paraformaldehyde. Levels of total Akt were measured using antibody ab81283 on an infrared in cell ELISA assay platform.



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

ab81283 staining AKT1 (phospho S473) in PC12 cells treated with galanin (1-29) (rat, mouse) (ab141153), by ICC/IF. Increase of AKT1 (phospho S473) expression correlates with increased concentration of galanin (1-29) (rat, mouse), as described in literature.
The cells were incubated at 37°C for 24h in media containing different concentrations of ab141153 (galanin (1-29) (rat, mouse)) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab81283 (1/100) dilution was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Western blot - Anti-AKT1 (phospho S473) [EP2109Y] antibody (ab81283)

Developed using the ECL technique

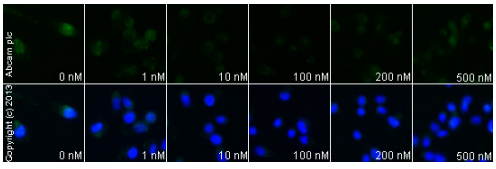
Performed under reducing conditions.

Predicted band size : 56 kDa
Observed band size : 60 kDa

Exposure time : 20 minutes

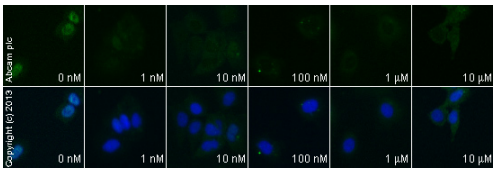
MCF7 cells were incubated at 37°C for 2 hours with vehicle control (0 µM) and different concentrations of CCCP (ab 141229). Increased expression of AKT1 (phospho S473) (ab81283) in MCF7 cells correlates with an increase in CCCP concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated withab81283at 2 µg/ml andab8227at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 and visualised using ECL development solution.



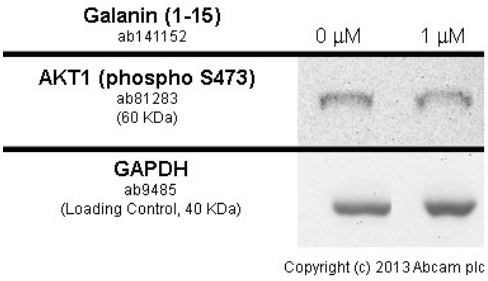
Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

ab81283 staining AKT1 (phospho S473) in PC3 cells treated with CAY10626 (ab120903), by ICC/IF. Decrease of AKT1 (phospho S473) expression correlates with increased concentration of CAY10626, as described in literature.
The cells were incubated at 37°C for 24h in media containing different concentrations of ab120903 (CAY10626) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab81283 (1/100) dilution was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

ab81283 staining AKT1 (phospho S473) in MCF7 cells treated with DAPT (ab120633), by ICC/IF. Decrease in expression of AKT1 (phospho S473) correlates with increased concentration of DAPT, as described in literature.
The cells were incubated at 37°C for 24h in media containing different concentrations of ab120633 (DAPT) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab81283 (1/200 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Western blot - Anti-AKT1 (phospho S473) [EP2109Y] antibody (ab81283)

Developed using the ECL technique

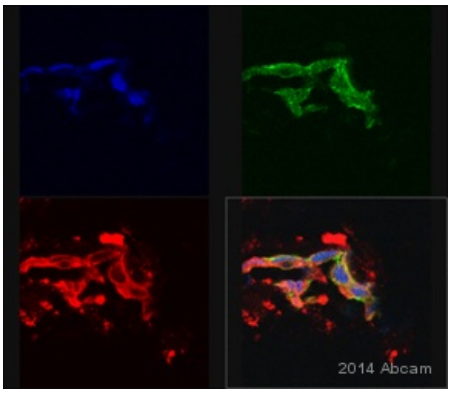
Performed under reducing conditions.

Predicted band size : 56 kDa
Observed band size : 60 kDa

Exposure time : 20 minutes

PC12 cells were incubated at 37°C for 24 hours with vehicle control (0 nM) and 1 µM of Galanin (1-15) (porcine, rat) (ab 141152). Decreased expression of AKT1 (phospho S473) (ab81283) in PC12 cells correlates with an increase in Galanin (1-15) (porcine, rat) concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 30µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated withab81283at 1 µg/ml andab8227at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 and visualised using ECL development solution.



Immunohistochemistry (Frozen sections) - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

This image is courtesy of an anonymous Abreview

ab81283 staining AKT1 (phospho S473) in Human peritoneal tumor tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with paraformaldehyde and blocked with 1% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (1/500) for 2 hours. An Alexa Fluor® 647-conjugated Donkey anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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 http://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