

Product datasheet

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

Recombinant RabMAb

★★★★☆ **10 Abreviews** **560 References** [14 Images](#)

Overview

Product name	Anti-AKT1 (phospho S473) antibody [EP2109Y]
Description	Rabbit monoclonal [EP2109Y] to AKT1 (phospho S473)
Host species	Rabbit
Specificity	AKT1 (phospho S473) antibody (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: Dot blot, In-Cell ELISA, IHC-P, WB Unsuitable for: Flow Cyt, ICC/IF or IP
Species reactivity	Reacts with: Mouse, Rat, Human, Recombinant fragment
Immunogen	Synthetic peptide corresponding to Human AKT1 (phospho S473). Database link: P31749 (Peptide available as ab171724 , ab217601)
Positive control	WB: HeLa (grown in serum free media overnight, then treated with 150nM Insulin for 5min) whole cell lysate; MCF7 (treated with CCCP) whole cell lysate; LNCaP (treated with 100nM Cacyculin A for 30min) whole cell lysate; NIH/3T3 (treated with PDGF) whole cell lysate; PC-12 whole cell lysate. IHC-P: Human cervical carcinoma tissue. Dot Blot: AKT1 (phospho S473) phospho peptide.
General notes	Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). See other anti-rabbit secondary antibodies that can be used with this antibody. 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 . We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified

format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

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	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2109Y
Isotype	IgG

Applications

The Abpromise guarantee 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
Dot blot		1/1000.
In-Cell ELISA		Use at an assay dependent concentration.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★★ (5)	1/5000 - 1/10000. Predicted molecular weight: 56 kDa. Abcam recommends using BSA as the blocking agent.

Application notes Is unsuitable for Flow Cyt, ICC/IF 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.

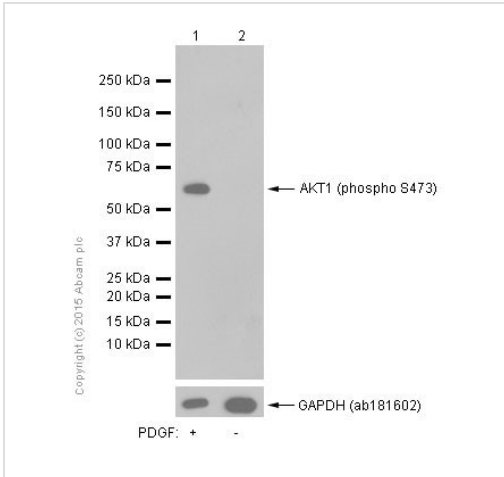
Post-translational modifications

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.

Images



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

All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 1/25000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) treated with 100ng/ml PDGF for 1 hour whole cell lysates

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

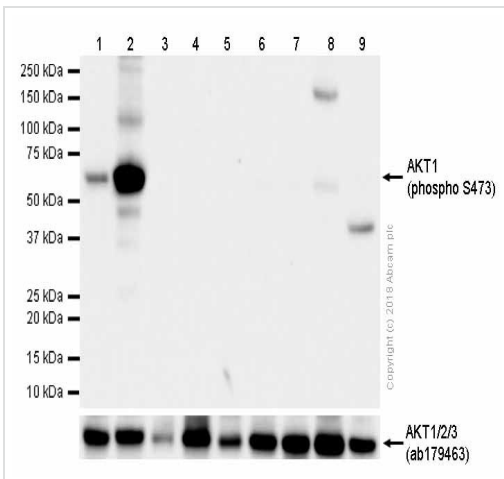
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 56 kDa

Observed band size: 56 kDa

Exposure time: 3 minutes

Blocking buffer and concentration: 5% NFDM/TBST



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

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

Lane 1 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysates

Lane 2 : LNCaP (Human prostate carcinoma epithelial cell) treated with 0.1 µM Calyculin A for 30 minutes whole cell lysates

Lane 3 : A549 (Human lung carcinoma epithelial cell) whole cell lysates

Lane 4 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 5 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates

Lane 6 : HUVEC (Human umbilical vein endothelial cell) whole cell lysates

Lane 7 : C2C12 (Mouse myoblasts myoblast) whole cell lysates

Lane 8 : Mouse brain lysates

Lane 9 : Rat heart lysates

Lysates/proteins at 20 µg per lane.

Secondary

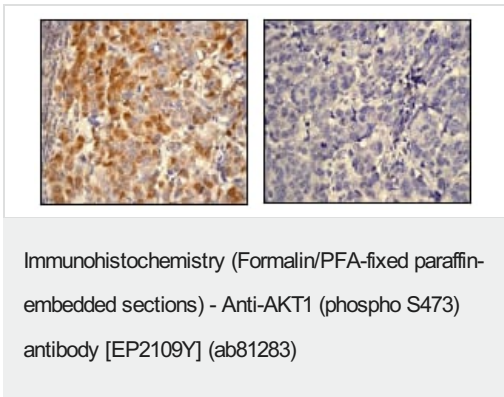
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 56 kDa

Exposure time: 50 seconds

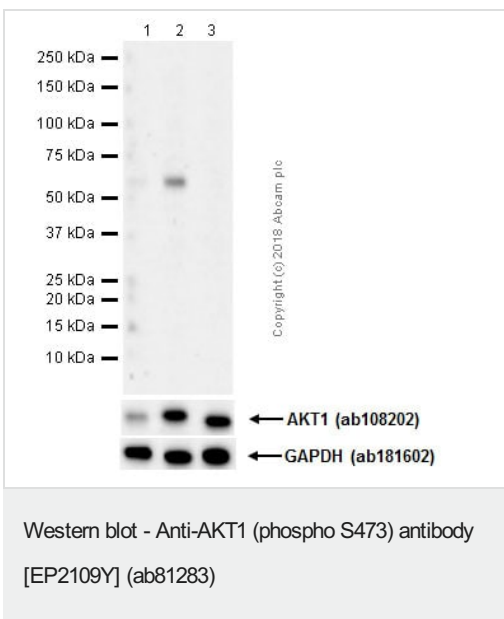
The basal expression level of AKT1 (phospho S473) varies in different cell lines reported by PMID: 19372546. But to detect clear signal, treatment is strongly recommended when using this antibody.

Blocking and diluting buffer: 5% NFDN/TBST



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

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 0.259 µg/ml

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) grown in serum free media overnight, whole cell lysate

Lane 2 : HeLa grown in serum free media overnight, then treated with 150nM Insulin for 5min, whole cell lysate

Lane 3 : HeLa grown in serum free media overnight, then treated with 150nM Insulin for 5min, whole cell lysate. Then the membrane was incubated with alkaline phosphatase

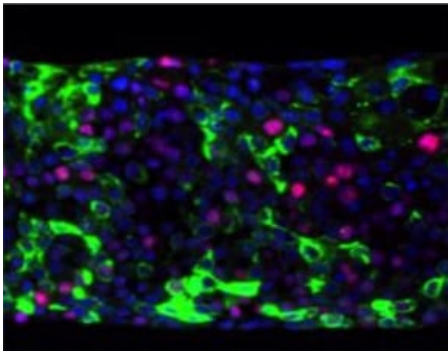
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 56 kDa

Blocking and diluting buffer: 5% NFDN/TBST.

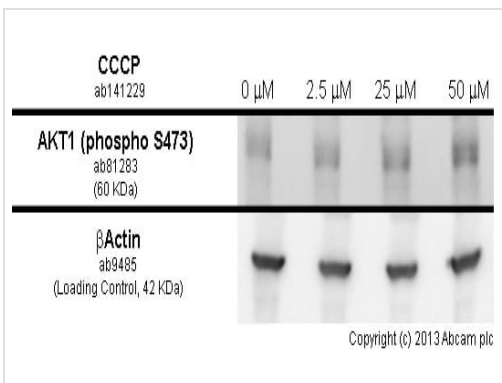


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

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). Fluorescein-conjugated tyramide was used to detect staining.

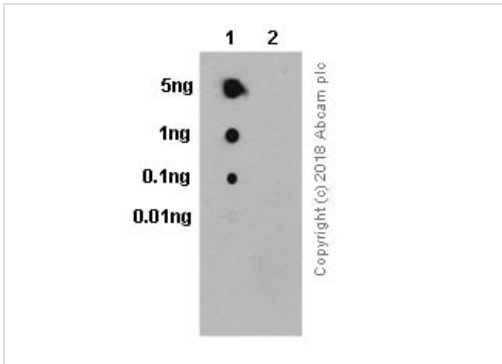


Western blot - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

MCF7 cells were incubated at 37°C for 2 hours with vehicle control (0 μ M) 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.

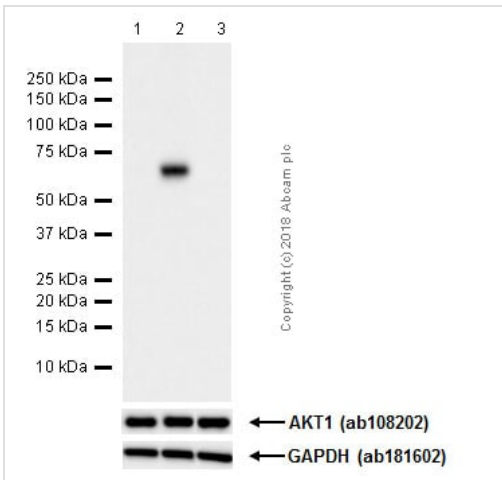
Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10 μ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab81283 at 2 μ g/ml and **ab8227** at 1 μ g/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.



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

Dot blot analysis of AKT1 (phospho S473) phospho peptide (Lane 1) and AKT1 non-phospho peptide (Lane 2) labelling AKT1 (phospho S473) phospho peptide with ab81283 at a dilution of 1:1000 dilution (0.259µg/ml). A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) was used as the secondary antibody at a dilution of 1:20,000 dilution.

Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM /TBST.



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

All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 0.259 µg/ml

Lane 1 : LNCaP (human prostate carcinoma epithelial cell) whole cell lysate

Lane 2 : LNCaP treated with 100nM Cacyculin A for 30min whole cell lysate

Lane 3 : LNCaP treated with 100nM Cacyculin A for 30min whole cell lysate. Then the membrane was incubated with alkaline phosphatase

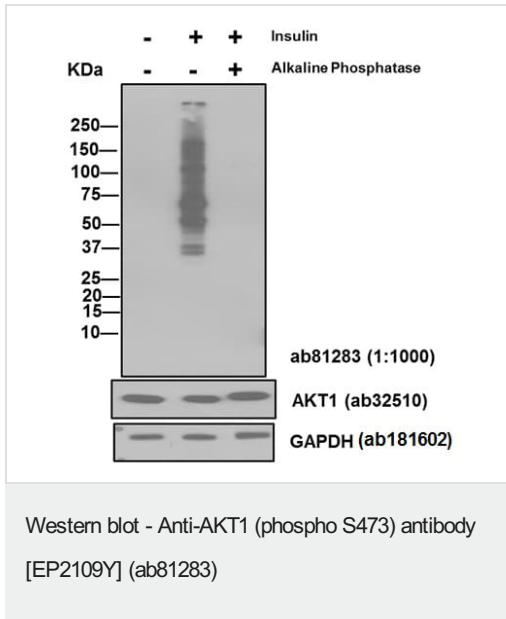
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 56 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



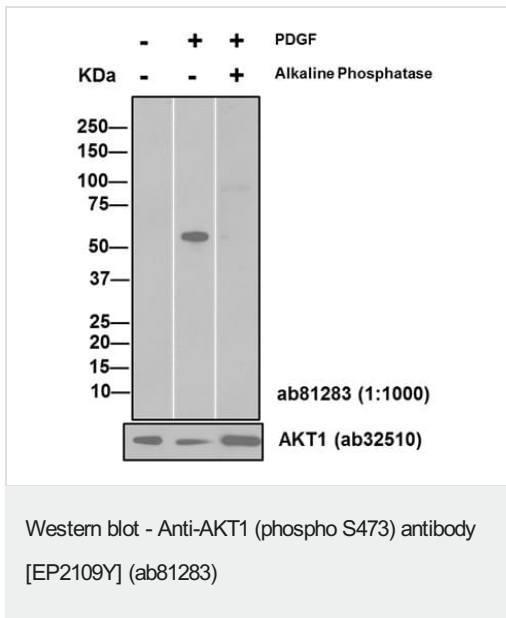
All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 1/1000 dilution

All lanes : 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.



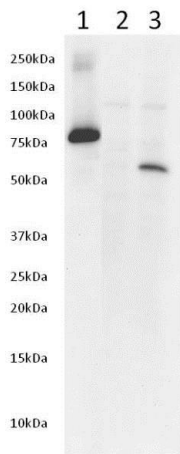
All lanes : Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283) at 1/1000 dilution

All lanes : NIH/3T3 cell lysate

Predicted band size: 56 kDa

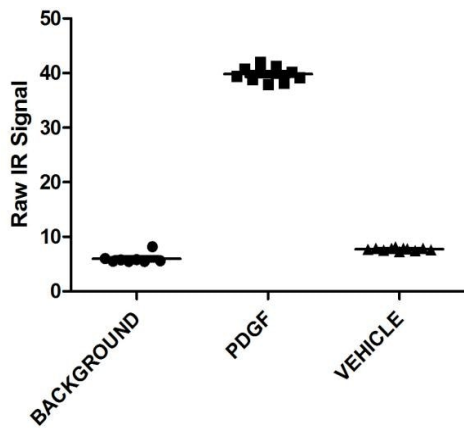
PDGF treatment: cells were starved overnight and then treated for 1 h with PDGF at 100 ng/ml.

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



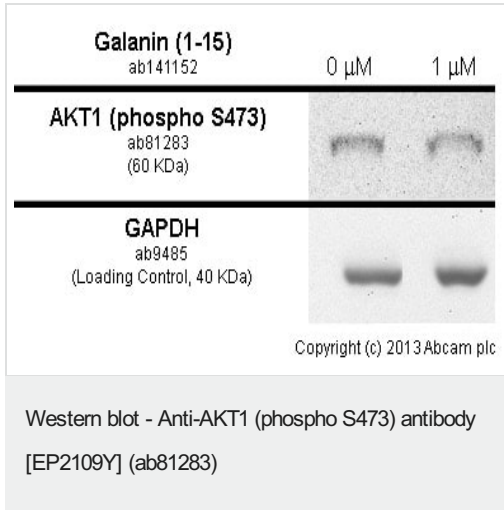
Western blot - Anti-AKT1 (phospho S473) antibody [EP2109Y] (ab81283)

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) antibody [EP2109Y] (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.



PC12 cells were incubated at 37°C for 24 hours with vehicle control (0 nM) and 1 μ M 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), 30 μ g 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 with ab81283 at 1 μ g/ml and ab8227 at 1 μ g/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.

Why choose a recombinant antibody?

 Research with confidence Consistent and reproducible results	 Long-term and scalable supply Recombinant technology
 Success from the first experiment Confirmed specificity	 Ethical standards compliant Animal-free production

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

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