



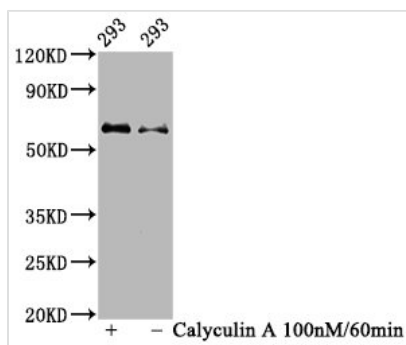
Phospho-AKT1 (Ser473) Recombinant Monoclonal Antibody

Product Code	CSB-RA001553A473phHU
Abbreviation	RAC-alpha serine/threonine-protein kinase
Storage	Upon receipt, store at -20°C or -80°C. Avoid repeated freeze.
Uniprot No.	P31749
Immunogen	A synthesized peptide derived from Human Phospho-AKT1 (Ser473)
Species Reactivity	Human
Tested Applications	ELISA, WB, IHC, IF, IP; Recommended dilution: WB:1:500-1:5000, IHC:1:50-1:200, IF:1:20-1:200, IP:1:200-1:1000
Relevance	<p>AKT1 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis. This is mediated through serine and/or threonine phosphorylation of a range of downstream substrates. Over 100 substrate candidates have been reported so far, but for most of them, no isoform specificity has been reported. AKT is responsible of the regulation of glucose uptake by mediating insulin-induced translocation of the SLC2A4/GLUT4 glucose transporter to the cell surface. Phosphorylation of PTPN1 at 'Ser-50' negatively modulates its phosphatase activity preventing dephosphorylation of the insulin receptor and the attenuation of insulin signaling. Phosphorylation of TBC1D4 triggers the binding of this effector to inhibitory 14-3-3 proteins, which is required for insulin-stimulated glucose transport. AKT regulates also the storage of glucose in the form of glycogen by phosphorylating GSK3A at 'Ser-21' and GSK3B at 'Ser-9', resulting in inhibition of its kinase activity. Phosphorylation of GSK3 isoforms by AKT is also thought to be one mechanism by which cell proliferation is driven. AKT regulates also cell survival via the phosphorylation of MAP3K5 (apoptosis signal-related kinase). Phosphorylation of 'Ser-83' decreases MAP3K5 kinase activity stimulated by oxidative stress and thereby prevents apoptosis. AKT 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. AKT is involved in the phosphorylation of members of the FOXO factors (Forkhead family of transcription factors), leading to binding of 14-3-3 proteins and cytoplasmic localization. In particular, FOXO1 is phosphorylated at 'Thr-24', 'Ser-256' and 'Ser-319'. FOXO3 and FOXO4 are phosphorylated on equivalent sites. AKT has an important role in the regulation of NF-kappa-B-dependent gene transcription and positively regulates the activity of CREB1 (cyclic AMP (cAMP)-response element binding protein). The phosphorylation of CREB1 induces the binding of accessory proteins that are necessary for the transcription of pro-survival genes such as BCL2 and MCL1. AKT phosphorylates 'Ser-454' on ATP citrate lyase (ACLY), thereby potentially regulating ACLY activity and fatty acid synthesis. Activates the 3B isoform of cyclic nucleotide phosphodiesterase (PDE3B) via</p>



phosphorylation of 'Ser-273', resulting in reduced cyclic AMP levels and inhibition of lipolysis. Phosphorylates PIKFYVE on 'Ser-318', which results in increased PI(3)P-5 activity. The Rho GTPase-activating protein DLC1 is another substrate and its phosphorylation is implicated in the regulation cell proliferation and cell growth. AKT plays a role as 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. 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). AKT mediates the antiapoptotic effects of IGF-I. Essential for the SPATA13-mediated regulation of cell migration and adhesion assembly and disassembly. May be involved in the regulation of the placental development. Phosphorylates STK4/MST1 at 'Thr-120' and 'Thr-387' leading to inhibition of its: kinase activity, nuclear translocation, autophosphorylation and ability to phosphorylate FOXO3. Phosphorylates STK3/MST2 at 'Thr-117' and 'Thr-384' leading to inhibition of its: cleavage, kinase activity, autophosphorylation at Thr-180, binding to RASSF1 and nuclear translocation. Phosphorylates SRPK2 and enhances its kinase activity towards SRSF2 and ACIN1 and promotes its nuclear translocation. Phosphorylates RAF1 at 'Ser-259' and negatively regulates its activity. Phosphorylation of BAD stimulates its pro-apoptotic activity. Phosphorylates KAT6A at 'Thr-369' and this phosphorylation inhibits the interaction of KAT6A with PML and negatively regulates its acetylation activity towards p53/TP53.

Form	Liquid
Conjugate	Non-conjugated
Storage Buffer	Rabbit IgG in phosphate buffered saline , pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Purification Method	Affinity-chromatography
Isotype	Rabbit IgG
Clonality	Monoclonal
Alias	RAC-alpha serine/threonine-protein kinase, Protein kinase B, PKB, Protein kinase B alpha, PKB alpha, Proto-oncogene c-Akt, RAC-PK-alpha, AKT1, PKB, RAC
Immunogen Species	Homo sapiens (Human)
Research Area	Signal Transduction
Gene Names	AKT1
Clone No.	4H12
Image	



Western Blot

Positive WB detected in 293 whole cell lysate(treated with Calyculin A or not)

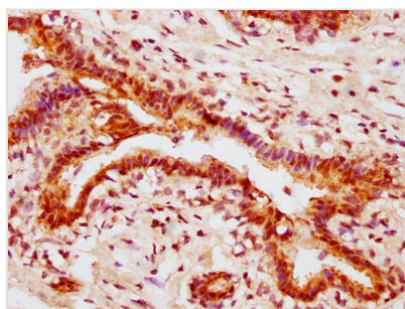
All lanes Phospho-AKT1 antibody at 1.08μg/ml

Secondary

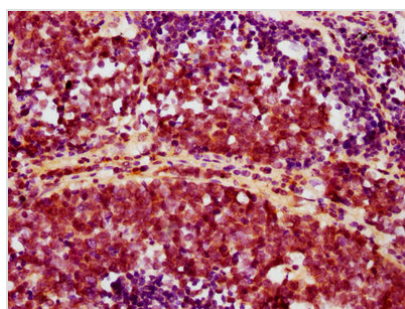
Goat polyclonal to rabbit IgG at 1/50000 dilution

Predicted band size: 60 KDa

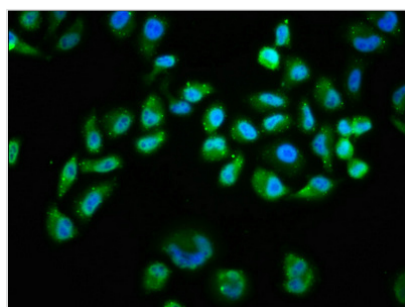
Observed band size: 60 KDa



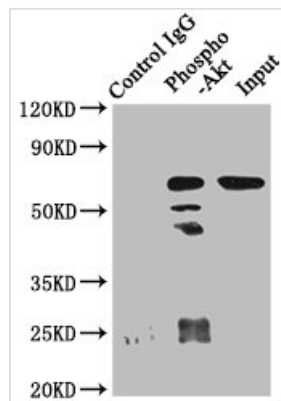
IHC image of CSB-RA001553A473phHU diluted at 1:100 and staining in paraffin-embedded human breast cancer performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4? overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.



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Immunofluorescence staining of HeLa cells(treated with 100mM EGF for 20min) with CSB-RA001553A473phHU at 1:68,counter-stained with DAPI. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and blocked in 10% normal Goat Serum. The cells were then incubated with the antibody overnight at 4?. The secondary antibody was Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L).



Immunoprecipitating Phospho-AKT1 in 293 whole cell lysate treated with Calyculin A

Lane 1: Rabbit control IgG(1 μ g)instead of CSB-RA001553A473phHU in 293 whole cell lysate treated with Calyculin A. For western blotting,a HRP-conjugated Protein G antibody was used as the secondary antibody (1/2000)

Lane 2: CSB-RA001553A473phHU(3 μ g)+ 293 whole cell lysate treated with Calyculin A(1mg)

Lane 3: 293 whole cell lysate treated with Calyculin A (20 μ g)

Description

The phospho-AKT1 (Ser473) recombinant monoclonal antibody is prepared through a combination of protein technology and DNA recombinant techniques. To begin, mice are immunized with a synthesized peptide derived from human phospho-AKT1 (Ser473), stimulating the production of B cells. Positive B cells are then selected and perform single clone identification. The resulting genes encoding the phospho-AKT1 (Ser473) antibody are amplified through PCR and inserted into a plasmid vector, creating a recombinant vector. This recombinant vector is introduced into host cells for antibody expression. The phospho-AKT1 (Ser473) recombinant monoclonal antibody is purified from the cell culture supernatant using affinity chromatography. It has been tested for use in five applications, including ELISA, WB, IHC, IF, and IP, and can react with human AKT1 protein phosphorylated at Ser473 residue.