abcam

Product datasheet

Human AKT1 ELISA Kit ab214023

Recombinant SimpleStep ELISA

4 Images

Overview

Precision

Product name Human AKT1 ELISA Kit

Detection method Colorimetric

Sample	n	Mean	SD	CV%
MCF7 extract	8			5.5%

Inter-assay

Intra-assay

Sample	n	Mean	SD	CV%
MCF7 extarct	3			7.9%

Sample type Cell culture extracts

Assay type Sandwich (quantitative)

Sensitivity 27.3 pg/ml

Range 187.5 pg/ml - 12000 pg/ml

Recovery Sample specific recovery

Sample type	Average %	Range
Cell culture extracts	97	96% - 98%

Assay time 1h 30m

Assay duration One step assay

Species reactivity Reacts with: Human

Product overview Human AKT1 ELISA Kit (ab214023) is a single-wash 90 min sandwich ELISA designed for the

> quantitative measurement of AKT1 protein in cell culture extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human AKT1 with 27.3 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This

approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

AKT1 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinases. These kinases regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis. The AKT kinases mediate these processes 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.

Each AKT family member contains an N-terminal pleckstrin homology (PH) domain, a central kinase domain, and a C-terminal regulatory domain. Mouse and rat AKT1 exhibit 98% amino acid identity to human AKT1.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Pre-coated microplate (12 x 8 well strips)

Notes

Platform

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human AKT1 Capture Antibody	1 x 600µl
10X Human AKT1 Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Human AKT1 Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit

Components	1 x 96 tests
Sample Diluent NS (ab193972)	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

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 (Pl(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

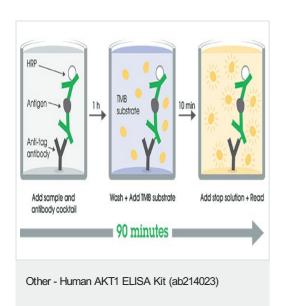
Phosphorylation on Thr-308, Ser-473 and Tyr-474 is required for full activity. Activated TNK2 phosphorylates it on Tyr-176 resulting in its binding to the anionic plasma membrane phospholipid PA. This phosphorylated form localizes to the cell membrane, where it is targeted by PDPK1 and PDPK2 for further 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.

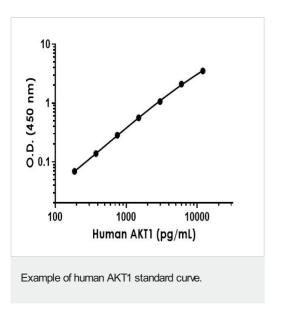
Cellular localization

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.

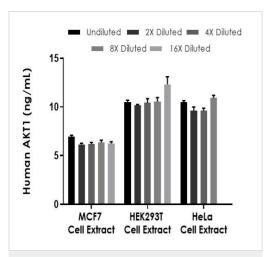
Images



SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

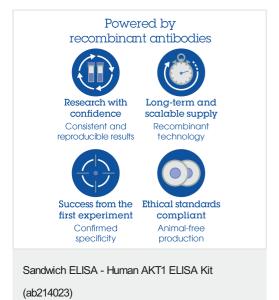


Background subtracted values are graphed. Data provided for demonstration purposes only.



Interpolated concentrations of native AKT1 in human MCF7, HEK 293T, and HeLa cell extract samples based on 75 µg/mL, 500 µg/mL, and 250 µg/mL extract loads, respectively.

The concentrations of AKT1 were measured in duplicate and interpolated from the AKT1 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean AKT1 concentration was determined to be 6,370.4 pg/mL, 10,792.3 pg/mL, and 10,157.2 pg/mL in MCF7, HEK 293T, and HeLa cell extract samples, respectively.



To learn more about the advantages of recombinant antibodies see **here**.

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