abcam

Product datasheet

AKT1 + AKT2 + AKT3 (pS473) ELISA Kit ab176635

SimpleStep ELISA

3 References 7 Images

Overview

Product name AKT1 + AKT2 + AKT3 (pS473) ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
HEK lysate	6			= 5.4%

Inter-assay

Sample	n	Mean	SD	CV%
HEK lysate	3			= 2.7%

Sample type Cell Lysate, Tissue Homogenate

Assay type Semi-quantitative

Sensitivity 30 pg/ml

Range 1 ng/ml - 100 ng/ml

Recovery 4.2 %

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Mouse, Human

Predicted to work with: Rat

Product overview Abcam's AKT 1/2/3 (pS473) *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of AKT 1/2/3 (pS473) protein in Human and mouse cells.

The SimpleStep ELISA™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound

material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

As of October 2019, this kit was reformulated with new antibodies to maintain continued long term supply.

Notes

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.

Platform

Microplate

Properties

Storage instructions

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

Components	1 x 96 tests
10X Wash Buffer PT	1 x 15ml
50X Cell Extraction Enhancer Solution	1 x 1ml
5X Cell Extraction Buffer PTR	1 x 12ml
AKT 1/2/3 (pS473) Capture Antibody	1 x 3ml
AKT 1/2/3 (pS473) Detector Antibody	1 x 3ml
Lyophilized AKT Control Lysate	1 vial
Plate Seal	1 unit
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Substrate	1 x 12ml

Function

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.

Tissue specificity

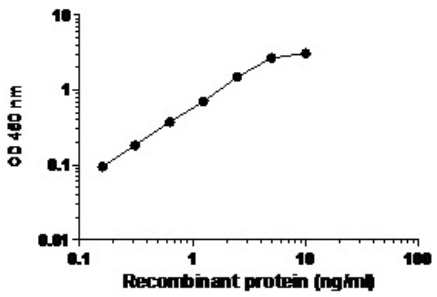
In adult tissues, it is highly expressed in brain, lung and kidney, but weakly in heart, testis and liver. In fetal tissues, it is highly expressed in heart, liver and brain and not at all in kidney.

Sequence similarities

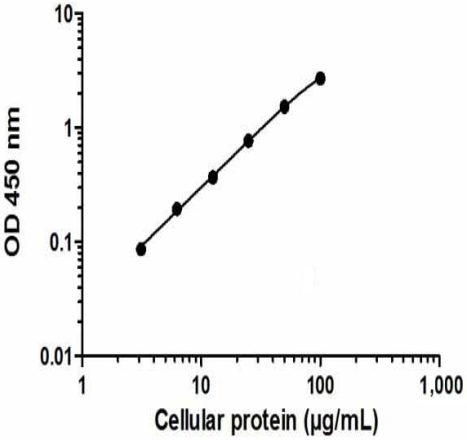
Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. RAC subfamily.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 PH domain.
Contains 1 protein kinase domain.

Domain	Binding of the PH domain to the phosphatidylinositol 3-kinase alpha (PI(3)K) results in its targeting to the plasma membrane.
Post-translational modifications	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.
Cellular localization	Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation.

Images



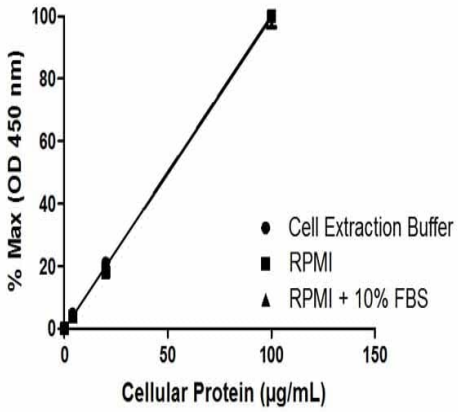
Typical Recombinant Protein Standard Curve



Typical cell lysate dilution series

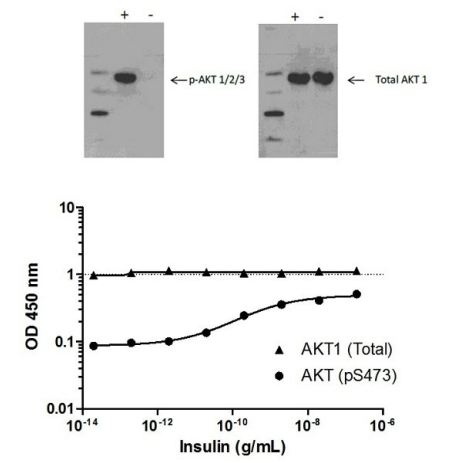
Example of a typical AKT (pS473) cell lysate dilution series.

Background-subtracted data values (mean +/- SD) are graphed.



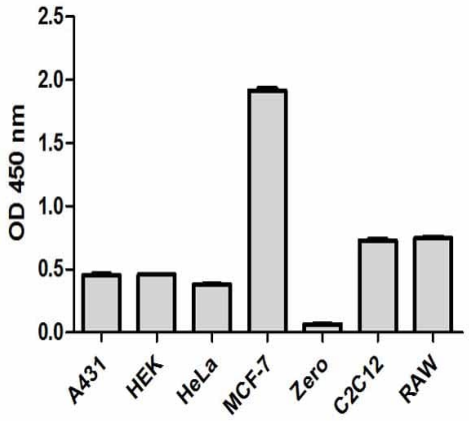
Linearity of dilution

Linearity of dilution in representative sample matrices. Cellular lysates were prepared at 3 concentrations in common media containing 1X Cell Extraction Buffer PTR. Data from duplicate measurements of AKT1 (pS473) are normalized and plotted.



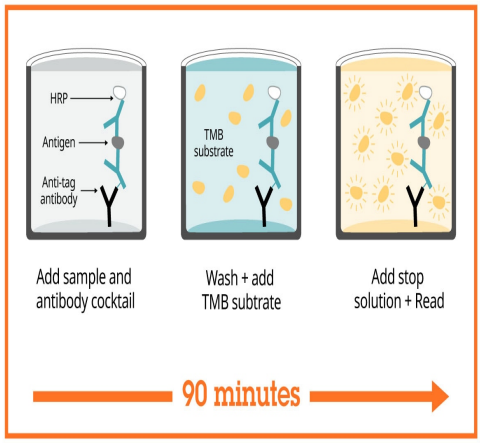
AKT (pS473) phosphorylation in response to insulin treatment.

Induction of AKT (pS473) phosphorylation in MCF-7 cells in response to insulin treatment. MCF-7 cells were cultured in 96-well tissue culture plates, serum-starved and treated (5 min) with a dose-range of insulin before cell lysis. Data from quadruplicate measurements of AKT (pS473) are plotted and compared against total AKT1 protein levels. Comparative AKT (pS473) and AKT1 (Total) data also shown by Western Blot.



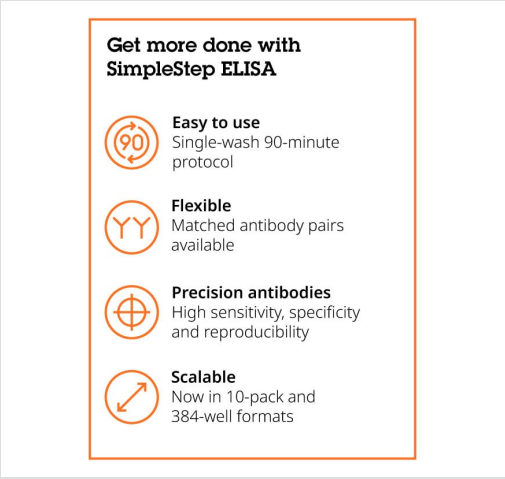
Comparison of total AKT1 expression in different cell lines

Cell line analysis for total AKT1 from 20 µg/mL preparations of cell extracts. Data from triplicate measurements (mean +/- SD) are plotted and compared to 1X Cell Extraction Buffer PTR (zero).



Sandwich ELISA - AKT1 + AKT2 + AKT3 (pS473) ELISA Kit (ab176635)

SimpleStep ELISA technology allows the formation of the antibody-antigen 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.



Sandwich ELISA - AKT1 + AKT2 + AKT3 (pS473) ELISA Kit (ab176635)

To learn more about the advantages of SimpleStep ELISA® kits see **here**.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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 **https://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