Datasheet for ABIN625220

AKT1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	AKT1
Binding Specificity:	pSer473
Reactivity:	Human, Mouse, Rat
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Phospho-Akt (pSer473) ELISA Kit for Semi-Quantitative measurement in cell lysates
Sample Type:	Cell Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human, mouse and rat Phospho-Akt (pSer473).
Components:	96 wells (12 strips x 8 wells) Coated With Antibody Wash Buffer Concentrate (20x) Positive Control Assay Diluent Detection Antibody Secondary Antibody or HRP Streptavidin TMB One-Step Substrate Reagent Stop Solution

Product Details

	Cell Lysis Buffer
Material not included:	Distilled or deionized water 100 mL and 1 liter graduated cylinders Tubes to prepare sample dilutions Protease and Phosphatase inhibitors Precision pipettes to deliver 2 µL to 1 mL volumes Adjustable 1-25 mL pipettes for reagent preparation Benchtop rocker or shaker Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	AKT1
Alternative Name:	Akt (AKT1 Products)
Pathways:	PI3K-Akt Signaling, RTK Signaling, TCR Signaling, AMPK Signaling, Interferon-gamma Pathway, TLR Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Response to Water Deprivation, Regulation of Actin Filament Polymerization , Carbohydrate Homeostasis, Glycosaminoglycan Metabolic Process, Cellular Glucan Metabolic Process, Regulation of Muscle Cell Differentiation, Cell-Cell Junction Organization, Regulation of Cell Size, Skeletal Muscle Fiber Development, Regulation of Carbohydrate Metabolic Process, Hepatitis C, Protein targeting to Nucleus, CXCR4-mediated Signaling Events, Signaling Events mediated by VEGFR1 and VEGFR2, Negative Regulation of intrinsic apoptotic Signaling, Thromboxane A2 Receptor Signaling, Signaling of Hepatocyte Growth Factor Receptor, Positive Regulation of fat Cell Differentiation, VEGFR1 Specific Signals, VEGF Signaling, Warburg Effect

Application Details

Comment:	Rapidly measure phosphorylated protein in lysates Screen numerous different cell lysates without performing a Western Blot analysis Minimal hands-on time, convenient, and non-radioactive material
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	Phospho-Akt (Ser473) ELISA (Enzyme-Linked Immunosorbent Assay) kit is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in human and mouse cell lysates. By determining phosphorylated Akt

	protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blotting analysis. This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of human, mouse and rat phospho-Akt (Ser473). An anti-pan Akt antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and Akt present in a sample is bound to the wells by the immobilized antibody. The wells are washed and anti-Akt (Ser473) antibody is used to detect phosphorylated Akt (Ser473). After washing away unbound antibody, HRP-conjugated anti-rabbit IgG is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Akt (Ser473) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. II.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use. 3. Preparation of Positive Control: Briefly spin the Positive Control vial of Item K. Add 500 µL 1x Assay Diluent (Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item K vial to prepare Positive Control (1) Solution (See i. Positive Control of part IX.for a typical result in page 9). Dissolve the powder thoroughly by a gentle mix (it can be removed by centrifuge if any precipitate in the solution is found). Pipette 300 µL 1x Assay Diluent into each tube. Use the Positive Control (1) to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background. Phospho-Akt (Ser473) ELISA Kit Protocol 6 4. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. 5. Briefly spin the detection antibody (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days or at - 80 °C for one month). The rabbit anti-Akt (Ser473) antibody should be diluted 55-fold with 1x Assay Diuent and used in step 4 of Part VII Assay Procedure. 6. Briefly spin the HRP-conjugated anti-rabbit IgG (Item D) before use. Pipette up and down to mix gently. HRP-conjugated anti- rabbit IgG concentrate should be diluted 500-fold with 1x Assay Diuent. For example: Briefly spin the vial (ItemD) and pipette up and down to mix gently. Add 10 µL of HRP-conjugated anti- P-1 P-2 P-3 P-4 0 150 µL Positive Control powder 500 µL 1x Assay Diluent 150µ l 150 µL Phospho-Akt (Ser473) ELISA Kit Protocol 7 rabbit IgG concentrate

	into a tube with 5 mL 1x Assay Diluent to prepare a 500-fold diluted HRP-conjugated anti-rabbit IgG solution. 7. Cell Lysate Buffer should be diluted 2-folds with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). VII.
Sample Preparation:	Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4 x 107 cells/mL in 1x Cell Lysate Buffer (we recommend adding protease and phosphatase inhibitors to Cell Lysate Buffer prior to sample preparation). Pipette up and down to resuspend and incubate the lysates with shaking at 2 - 8° C for 30 minutes. Microcentrifuge at 13,000 rpm for 10 minutes at 2 - 8° C, and transfer the supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at -70 °C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use. For the initial experiment, we recommend to do a serial dilution testing such as 5-fold and 50-fold dilution for your cell lysates with 1x Assay Diluent (Item E) before use. Phospho-Akt (Ser473) ELISA Kit Protocol 5 Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empirically. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors).
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate. 2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of prepared 1x detection antibody anti-Akt (Ser473) (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. Phospho-Akt (Ser473) ELISA Kit Protocol 8 5. Discard the solution. Repeat the wash as in step3. 6. Add 100 µL of prepared 1x HRP-conjugated anti-rabbit IgG (see Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with shaking. 7. Discard the solution. Repeat the wash as in step3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30

Application Details

	minutes at room temperature in the dark with shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	ELISA data analysis: Average the duplicate readings for each sample or positive. i. Positive Control A431 cells were treated with recombinant human EGF at 37 °C for 20 min. Solubilize cells at 4 x 107 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI Reagent Preparation for detail. Assay Diluent Positive control dilution series O D = 4 5 0 n m 0.1 1 P-1 P-2 P-3 P-4 0 - Phospho-Akt (Ser473) ELISA Kit Protocol 10 ii. Recombinant Human PDGF Stimulation of NIH3T3 Cell Lines NIH3T3 cells were treated or untreated with recombinant human PDGF for 10 min. Cell lysates were analyzed using this phosphoELISA and Western Blot. A). ELISA Phospho-Akt (Ser473) Pan Akt O D =4 50 n m 0.0 0.5 1.0 1.5 2.0 2.5 3.0 NIH3T3 NIH3T3+PDGF B). Western-Blot Analysis PDGF 0 10 0 10 (Min) Anti-phospho-Akt Anti-pan Akt (Ser473) Phospho-Akt (Ser473) ELISA Kit Protocol 11 iii. SENSITIVITY The NIH3T3 cells were treated with recombinant human PDGF for 10 minutes to induce phosphorylation of Akt. Serial dilutions of lysates were analyzed in this ELISA and by Western blot. Immunoblots were incubated with anti-phospho-Akt (Ser473). A) ELISA 40 13.3 4.4 1.48 0.49 0 (µg) O D =4 50 n m 0.0 0.2 0 4 0.6 0.8 1.0 1.2 1.4 1.6 X
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze- thaw cycles.
Storage:	4 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months

Publications

Product cited in:	Sharma, Yang, Xi, Grotta, Aronowski, Savitz: "IL-10 directly protects cortical neurons by activating PI-3 kinase and STAT-3 pathways." in: **Brain research**, Vol. 1373, pp. 189-94, (2011) (

Publications

PubMed).

Images

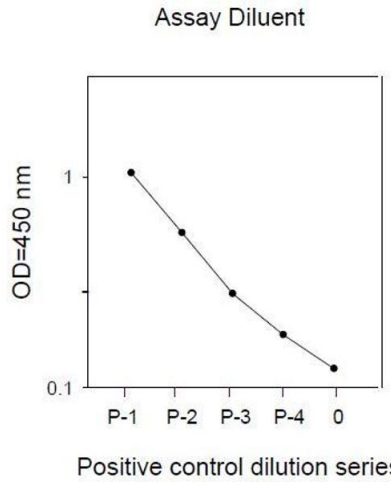

Image 1.

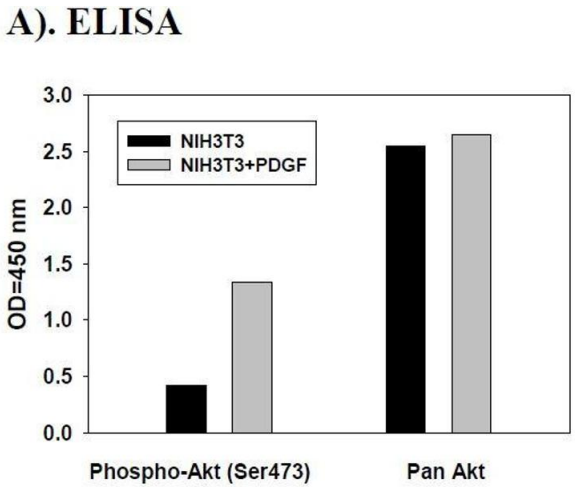

Image 2.

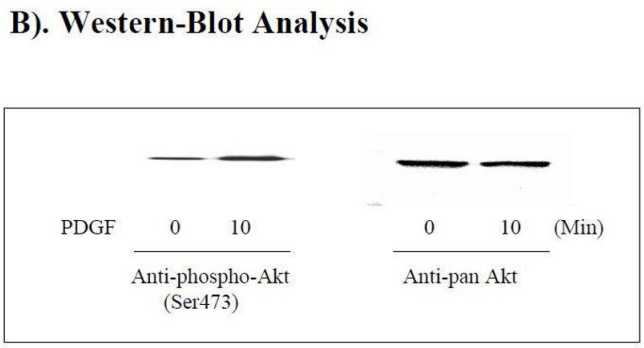

Image 3.

Please check the product details page for more images. Overall 5 images are available for ABIN625220.