abcam

Product datasheet

Akt Kinase Activity Assay Kit ab139436

9 References 1 Image

Overview

Product name

Detection method

Precision

Akt Kinase Activity Assay Kit

Colorimetric

SD CV% Sample Mean n Sample < 10%

Inter-assay

Intra-assav

Sample	n	Mean	SD	CV%
Sample				< 10%

Sample type

Assay type

Product overview

Adherent cells, Suspension cells, Tissue Extracts, Purified protein

Enzyme activity

Akt Kinase Activity Assay Kit (ab139436) is a non-radioactive assay providing a safe, rapid and reliable method for the screening of inhibitors or activators of Akt and for quantitating the activity of Akt in purified or partially purified enzyme preparations. This kit is based on a solid phase enzyme-linked immuno-absorbent assay (ELISA) that utilizes a specific synthetic peptide as a substrate for Akt and a polyclonal antibody that recognizes the phosphorylated form of the substrate. The assay is designed for the analysis of Akt activity in the solution phase. For the measurement of Akt in partially purified and purified enzyme preparations from any species.

The kit offers the following advantages:

- 1. Safe non-radioactive measurement of kinase activity
- 2. Flexible kinetic and end-point options available
- 3. Fast results in < 4.5 hours
- 4. Efficient only 30 µl diluted sample needed per well

Microplate reader

Platform

Properties

Storage instructions

Please refer to protocols.

Components	1 x 96 tests
20X Wash Buffer	1 x 30ml
Active Akt	1 x 30µl
Akt Phosphospecific Substrate Antibody	1 x 5ml
Akt Substrate Microtiter Plate	1 unit
Antibody Dilution Buffer	1 x 10ml
Anti-Rabbit lgG: HRP Conjugate	1 x 20µl
ATP	1 x 2mg
Kinase Assay Dilution Buffer	1 x 10ml
Stop Solution 2	1 x 10ml
TMB Substrate	1 x 10ml

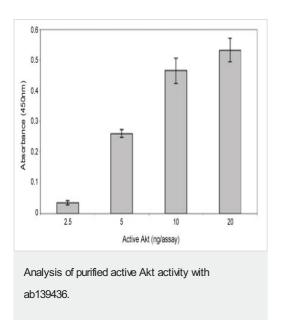
Relevance

AKT, also known as protein kinase B (PKB), is a serine/threonine protein kinase. There are three mammalian isoforms of AKT: AKT1 (PKB alpha), AKT2 (PKB beta) and AKT3 (PKB gamma) with AKT2 and AKT3 being approximately 82% identical with the AKT1 isoform. Each isoform has a pleckstrin homology (PH) domain, a kinase domain and a carboxy terminal regulatory domain. AKT was originally cloned from the retrovirus AKT8, and is a key regulator of many signal transduction pathways. Its tight control over cell proliferation and cell viability are manifold; overexpression or inappropriate activation of AKT has been seen in many types of cancer. AKT mediates many of the downstream events of phosphatidylinositol 3 kinase (a lipid kinase activated by growth factors, cytokines and insulin). Pl3 kinase recruits AKT to the membrane, where it is activated by PDK1 phosphorylation. Once phosphorylated, AKT dissociates from the membrane and phosphorylates targets in the cytoplasm and the cell nucleus. AKT has two main roles: (i) inhibition of apoptosis; (ii) promotion of proliferation. AKT has been shown to play a role in such metabolic processes as glucose transport, glycogen synthesis, glycolysis, and protein synthesis. It had also been shown to promote cell survival by inhibiting apoptosis through its ability to phosphoylate and inactivate several targets, including Bad, Forkhead transcription factors, and caspase 9. Activity of AKT has been associated with the phosphorylation of two sites: T308, in the activation loop of the kinase, and S473, at the carboxyl terminus. Phosphorylation of both sites contributes to AKT activity, however phosphorylation of T308 has been shown to be absolutely essential for AKT activation.

Cellular localization

Cell Membrane, Cytoplasmic and Nuclear. Note=Nucleus after activation by integrin-linked protein kinase 1 (ILK1).

Images



Varying quantities of purified active Akt were assayed using the Akt substrate microtiter plate. Assays were incubated for 60 minutes at 30°C.

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