

ab65786

Akt Activity Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Akt Activity in various samples.

This product is for research use only and is not intended for diagnostic use.

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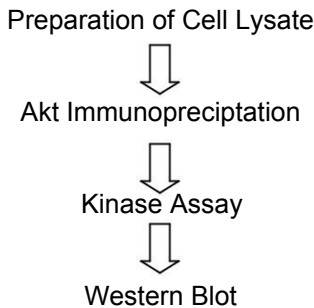
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1. Overview

Akt is a protein kinase that can be activated by insulin and various growth factors and functions in a pathway involving PI3 kinase. Recent evidence suggests that Akt functions to promote cell survival by actively inhibiting apoptosis.

Abcam's Akt Activity Assay Kit utilizes an Akt-specific antibody to immunoprecipitate Akt from cell lysate. Activity of the Akt is then determined in a kinase reaction using recombinant GSK-3 α as substrate. Phosphorylation of the GSK-3 α can be analyzed by Western blot analysis using the phospho-GSK-3 α specific antibody included in the kit. The kit specifically detects Akt1, Akt2, and Akt3 activities, other kinase activities would not be detected.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Kinase Extraction Buffer	80 mL
Akt Specific Antibody	80 μ L
Protein A Sepharose	2 mL
GSK-3 α Protein/ATP Mixture	80 μ L
Kinase Assay Buffer	25 mL
Phospho-GSK-3 α Specific Antibody	50 μ L

* Store kit at -20°C.

B. Additional Materials Required

- Enzyme or fluorophore-conjugated anti-rabbit IgG secondary antibody - for WB detection of rabbit anti-Phospho-GSK-3 α (Ser 21) Specific Antibody
- 1X PBS
- 3X SDS-PAGE Buffer

4. Assay Protocol

1. Preparation of Cell Lysate:

- a) Activate cells by desired methods. Concurrently incubate a control culture *without* activation.

Note:

To generate a positive control, cells can be starved (serum-free) for 3 hours, and then added 20% serum back for 30 min before collected.

- b) Pellet cells ($2 \cdot 10^6$ /assay) and wash once in 1X ice-cold PBS.
- c) Lyse cells in 200 μ l ice-cold Kinase Extraction Buffer. Incubate on ice for 5 min.
- d) Pellet at 13,000 rpm for 10 min at +4°C. Transfer supernatant (i.e. the Cell Lysate) to a new tube.
- e) Assay protein concentration of the Cell Lysate. The Cell Lysate can be used immediately or freeze at -80°C for future use.

2. Akt Immunoprecipitation:

- a)** For each assay, add 2 μ l Akt Specific Antibody (reacts with human, mouse, and rat) to 200 μ l Cell Lysate (~50-400 μ g total protein), and rotate for 45 min at room temperature.
- b)** Re-suspend Protein A sepharose by gently vortex to a slurry form. Add 50 μ l of the Protein A-Sepharose slurry to each sample and continue rotating for 1 hour at room temperature.
- c)** Centrifuge at 15,000 rpm for 2 min, remove supernatant.
- d)** Wash the protein A beads two times with 0.5 ml Kinase Extraction Buffer and one time with 0.5 ml Kinase Assay Buffer.

3. Kinase Assay:

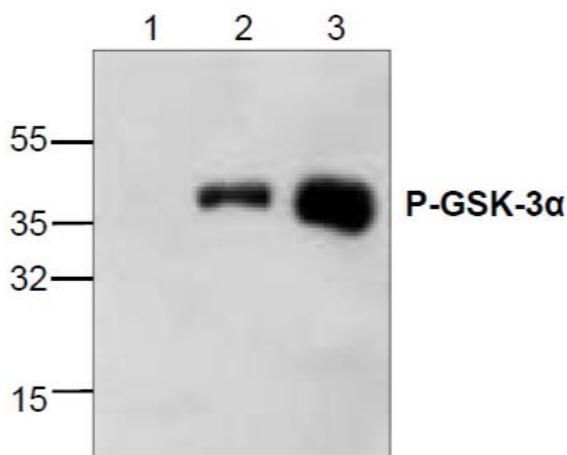
- a)** Add 50 μ l Kinase Assay Buffer to the washed Protein A beads, add 2 μ l GSK-3 α Protein/ATP Mixture and incubate at 30°C for 1-4 hr.
- b)** Spin down the Protein A beads, collect 30 μ l supernatant into a new eppendorf tube. Add 15 μ l 3X SDS-PAGE Buffer (not provided) to 30 μ l supernatant. Discard the protein A beads.
- c)** Boil the 45 μ l supernatant (containing SDS PAGE buffer) for 3 min. Microcentrifuge for 2 min to spin down any Protein A Beads that may have been transferred in step 3b and extract the supernatant.
- d)** Load the supernatant (20 μ l) on a 12% SDS-PAGE gel. Alternatively, the supernatant may be stored at -20°C for future use.

4. Western Blot:

Perform Western blotting using the rabbit anti-Phospho-GSK-3 α (Ser 21) Specific Antibody at 1:1000 dilutions.

A 37 kDa band corresponding to the phosphorylated GSK-3 α should be detected in Akt activated samples.

5. Data Analysis



Western blot analysis of phospho-GSK-3 α in Akt Negative (Lane 1) and Akt positive (Lane 2, 3) Jurkat cell lysates.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).

Version 3a

Notes:

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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