RayBio® KinaseSTAR™ Akt Activity Assay Kit

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RayBio® KinaseSTAR™ Akt Activity Kit Protocol

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RayBiotech, Inc.

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Tel:(Toll Free)1-888-494-8555 or 770-729-2992; Fax:770-206-2393;
Web: www.raybiotech.com Email: info@raybiotech.com

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I. INTRODUCTION

Akt is a protein kinase that can be activated by insulin and various growth factors and functions in the PI3 kinase pathway. Recent evidence suggests that Akt functions to promote cell survival by actively inhibiting apoptosis. The Akt Activity Assay Kit utilizes an Akt-specific antibody to immunoprecipitate Akt from cell lysate samples. Akt activity is then determined via a kinase reaction using recombinant GSK-3α as an enzyme substrate. Phosphorylation of the GSK-3α can then be analyzed by Western blot analysis using the included phospho-GSK-3α specific antibody. The kit specifically detects Akt1, Akt2, and Akt3 enzyme activities, other kinase activities would not be detected.

II. REAGENTS

Components	ASP-S100	Cap Code	Part Number
Kinase Extraction Buffer	80 ml	NM	Item A
Akt Specific Antibody	80 µl	Red	Item B
Protein A Sepharose	2 ml	Clear	Item C
GSK-3α Protein/ATP Mixture	80 µl	Blue	Item D
Kinase Assay Buffer	25 ml	WM	Item E
Phospho-GSK-3α Specific Antibody	50 µl	Green	Item F

III. AKT ACTIVITY ASSAY PROTOCOL

A. Preparation of Cell Lysate:

1. Activate cells by desired methods. Incubate a control sample separately that is not activated. Note: To generate a positive control, cells can be serum-starved for 3 hrs, and then have 20% serum back added back 30 min before sample collection.
2. Pellet cells (2-10^6/assay) and wash once in 1X ice-cold PBS.
3. Lyse cells in 200 µl ice-cold Kinase Extraction Buffer. Incubate on ice for 5 min.
4. Pellet at 13,000 rpm for 10 min at 4°C. Transfer supernatant (This is the Cell Lysate) to a new tube.
5. Assay protein concentration of the Cell Lysate. The Cell Lysate can be used immediately or freeze at -80°C for future use.
    a. BCA assays like 68QT-BCAPro-S1000, are recommended for protein quantification

B. Akt Immunoprecipitation:

1. For each assay, add 2 µl Akt Specific Antibody (reacts with human, mouse, and rat) to 200 µl Cell Lysate (~50-400 µg total protein), and rotate or rock the sample slowly for 45 min at room temperature.

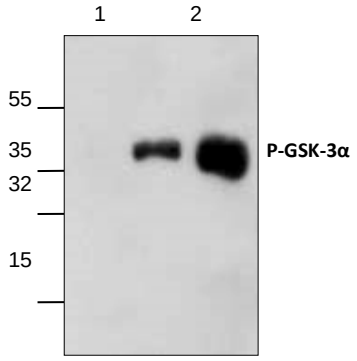
2. Resuspend Protein A sepharose by gentle vertex to create a slurry. Add 50 µl of the Protein A Sepharose slurry to each sample and continue to rock gently for 1 hour at room temperature.
3. Centrifuge at 15,000 rpm for 2 min, remove supernatant.
4. Wash the protein A beads two times with 0.5 ml Kinase Extraction Buffer and one time with 0.5 ml Kinase Assay Buffer.

C. Kinase Assay:

1. Add 50 µl Kinase Assay Buffer to the washed Protein A beads, then add 2 µl GSK-3α Protein/ATP Mixture and incubate at 30°C for 1-4 hr.
2. Spin down the Protein A beads (15,000 rpm for 2 min) then collect 30 µl of supernatant into a new eppendorf tube. Add 15 µl 3X SDS-PAGE Buffer (not provided).
3. Boil the samples for 3 min and then centrifuge (15,000 rpm for 2 min) to spin down the Protein A Beads.
4. Load the supernatant (20 µl) onto a 12% SDS-PAGE gel. Alternatively, the supernatant may be stored at -20°C for future use.

D. Western Immunoblotting:

1. Perform Western blotting using the rabbit anti-Phospho-GSK-3α (Ser 21) specific Antibody at 1:1000 dilutions. A 37 kDa band corresponding to the phosphorylated GSK-3α should be detected in Akt activated samples.



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

This product is for research use only.