KinaseSTAR™ Akt Activity Assay Kit

(Catalog #K435-40; 40 assays; Store kit at –20˚C)

I. Introduction:

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. The 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-3α as substrate. Phosphorylation of the GSK-3α can be analyzed by Western blot analysis using the phospho-GSK-3α specific antibody included in the kit. The kit specifically detects Akt1, Akt2, and Akt3 activities, other kinase activities would not be detected.

II. Kit Contents:

Component	K435-40 Part No.	K435-40 40 assays	Color Code Cap Color
Kinase Extraction Buffer	K435-40-1	80 ml	NM
Akt Specific Antibody	K435-40-2	80 µl	Red
Protein A Sepharose	K435-40-3	2 ml	Clear
GSK-3α Protein/ATP Mixture	K435-40-4	80 µl	Blue
Kinase Assay Buffer	K435-40-5	25 ml	WM
Phospho-GSK-3α Specific Antibody	K435-40-6	50 µl	Green

III. Akt Activity Assay Protocol

A. Preparation of Cell Lysate:

1. 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 hrs, and then added 20% serum back for 30 min before collected.
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.

B. Akt Immunoprecipitation:

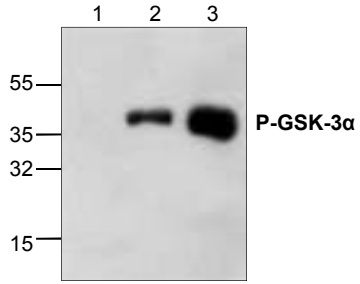
6. For each assay, add 2 µl Akt Specific Antibody (reacts with human, mouse, and rat) to 200 µl Cell Lysate (~50-400 µg total protein), and rotate for 45 min at room temperature.
7. Resuspend Protein A sepharose by gently vertex to a slurry form. Add 50 µl of the Protein A-Sepharose slurry to each sample and continue rotating for 1 hour at room temperature.
8. Centrifuge at 15,000 rpm for 2 min, remove supernatant.
9. 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:

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

D. Western Immunolotting:

14. 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.



Cat. #:K435-40
Western blot analysis of phosphor-GSK-3α in Akt Negative (Lane 1, Cat.# 7035) and Akt positive (Lane 2,3, Cat.# 7036) Jurkat cell lysates.

IV. Related Products:

- Akt Positive & Negative cell Lysates
- Akt Polyclonal Antibodies
- Akt Inhibitors
- Active Akt Proteins
- Other Kinase Assay Kit and Reagents (many)
- Apoptosis & Cell Proliferation Assays
- Metabolism & Obesity Related Assay Kits