

# 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-3 $\alpha$  as substrate. Phosphorylation of the GSK-3 $\alpha$  can be analyzed by Western blot analysis using the phospho-GSK-3 $\alpha$  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	K435-40	Color Code
	Part No.	40 assays	Cap Color
Kinase Extraction Buffer	K435-40-1	80 ml	NM
Akt Specific Antibody	K435-40-2	80 $\mu$ l	Red
Protein A Sepharose	K435-40-3	2 ml	Clear
GSK-3 $\alpha$ Protein/ATP Mixture	K435-40-4	80 $\mu$ l	Blue
Kinase Assay Buffer	K435-40-5	25 ml	WM
Phospho-GSK-3 $\alpha$ Specific Antibody	K435-40-6	50 $\mu$ l	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 millions/assay) and wash once in 1X ice-cold PBS.
3. Lyse cells in 200  $\mu$ l ice-cold Kinase Extraction Buffer. Incubate on ice for 5 minutes.
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  $\mu$ l Akt Specific Antibody (reacts with human, mouse, and rat) to 200  $\mu$ l Cell Lysate (~50-400  $\mu$ g total protein), and rotate for 45 min at room temperature.

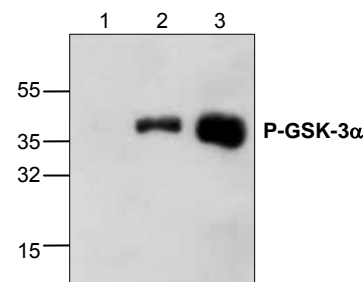
7. Resuspend Protein A sepharose by gently vortex to a slurry form. Add 50  $\mu$ l of the Protein A-Sepharose slurry to each sample and continue rotating for 1 hour at room temperature.
8. Centrifuge at 15,000 rpm for 2 min, remove supernatant.
9. Wash the protein A beads two times with 0.5 ml Kinase Extraction Buffer and one time with 0.5 ml Kinase Assay Buffer.

### C. Kinase Assay:

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

### D. Western Immunoblotting:

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



**Cat. #:K435-40**

Western blot analysis of phospho-GSK-3 $\alpha$  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