Product Information

KinaseSTAR Akt Activity Assay Kit

I. Kit Contents:

Components	K2080-40 40 assays	Cap Color	Part Number
Kinase Extraction Buffer	80 ml	NM	K2080-C-1
Akt Specific Antibody	80 µl	Red	K2080-C-2
Protein A Sepharose	2 ml	Clear	K2080-C-3
GSK-3α Protein/ATP Mixture	80 µl	Blue	K2080-C-4
Kinase Assay Buffer	25 ml	WM	K2080-C-5
Phospho-GSK-3α Specific Antibody	50 µl	Green	K2080-C-6

II. Introduction:

Akt, also known as protein kinase B, is a serine/threonine-specific protein kinase that can be activated by insulin and various growth factors. Akt plays important roles in multiple cellular processes such as apoptosis, cell proliferation, glucose metabolism, transcription and cell migration. Akt is involved in PI3 kinase pathway.

The KinaseSTAR Akt Activity Assay Kit provides a specific and simple way for detection of Akt activity based on Western Blot method. The assay uses an Akt-specific antibody to immunoprecipitate Akt from tissue or cell lysates. The kinase reaction conducted to detect Akt activity is performed using recombinant GSK-3α as substrate. The phosphorylation of GSK-3α is analyzed by Western blot method using a phospho-GSK-3α specific antibody. The kit specifically detects Akt1, Akt2, and Akt3 activities, without other kinase activity can be detected.

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℃. 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℃ 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℃ 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℃ 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.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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