KinaseSTAR™ JNK Activity Screening Kit

(Catalog #K430-40; 40 assays; Store kit at –20˚C)

I. Introduction:

c-Jun N-terminal kinase (JNK) is one of the several main MAP kinase groups identified in mammals. Recent evidences suggest that activation of JNK may play an important role in neuronal apoptosis and other physiological and pathological processes. The JNK Activity screening kit utilizes an N-terminal c-Jun (1-79) fusion protein bound to glutathione sepharose beads to selectively "pull down" JNK from cell lysates. After washing to remove nonspecifically bound proteins, the kinase reaction is then carried out in the presence of cold ATP. c-Jun phosphorylation is then measured by Western blot analysis using a phospho-c-Jun specific antibody.

II. Kit Contents:

Component	K430-40	Cap Color
Kinase Extraction Buffer	80 ml	NM
c-Jun (1-79) Fusion Protein Beads	800 µl	Red
Kinase Assay Buffer	25 ml	WM
ATP (10 mM)	50 µl	Yellow
Phospho-cJun Specific Antibody	50 µl	Green

III. JNK Activity Immunoassay Protocol

A. Preparation of Cell Lysate:

1. Activate cells by desired methods. Concurrently incubate a negative control culture without activation. To generate a positive control, cells can be treated with 1 µg/ml of Anisomycin (Cat.# 1549-10) for 1 hr, before harvested.
2. Pellet cells (2-10 millions/assay) and wash once in 1X ice-cold PBS.
3. Lyse cells in 200 µl ice-cold JNK 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. "Pull Down" JNK Using c-Jun Fusion Protein:

6. For each assay, add 20 µl c-Jun Fusion Protein Beads to 200 µl Cell Lysate (~50-400 µg total protein). Incubate with gentle rocking overnight at 4˚C.
7. Microcentrifuge (14,000 rpm) for 30 sec at 4˚C. Remove Supernatant. Wash pellet twice with 0.5 ml of 1X Kinase Extraction Buffer and one time with 0.5 ml Kinase Assay Buffer. Keep on ice.

C. Kinase Assay:

8. Suspend pellet in 50 µl Kinase Assay Buffer. Add 1 µl of 10 mM ATP. Incubate for 30 min at 30°C.
9. Add 30 µl 3X SDS-PAGE Buffer (not provided)
10. Boil the samples for 3 min. Microcentrifuge for 2 min.
11. Load the supernatant (20 µl) on 12% SDS-PAGE. Alternatively, the supernatant may be stored at -20°C for future use.

D. Western Immunoblotting:

12. Perform Western blot analysis using the rabbit anti-Phospho-cJun (Ser 73) Specific Antibody at 1:1000 dilution. A 35 kDa band corresponding to the phosphorylated c-Jun protein should be detected in JNK activated samples.

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