Introduction and Background

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 glutathion sepharose beads to selectively "pull down" JNK from cell lysates. After wash 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.

Material and Method

A. List of component

1. Kinase Extraction Buffer: 80 ml.
2. c-Jun (1-79) Fusion Protein Beads: 800 µl.
3. Kinase Assay Buffer: 25 ml.
4. ATP (10 mM): 50 µl.
5. Phospho-cJun Specific Antibody: 50 µl.

B. 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 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 minutes.
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.

C. "Pull Down" JNK Using c-Jun Fusion Protein

1. 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℃.
2. Microcentrifuge (14,000 rpm) for 30 seconds at 4℃. 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.

D. Kinase Assay

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

for future use.

E. Western Immunolotting

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