BioVision

Rev. 05/11

For research use only

KinaseSTAR^{TM} JNK Activity Assay Kit

(Catalog #K431-40; 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 evidence suggest that activation of JNK may play an important role in neuronal apoptosis and other physiological and pathological processes. The JNK Activity Immunoassay Kit utilizes a JNK-specific antibody to immunoprecipitate JNK from cell lysate. Activity of the JNK is then determined in a kinase reaction using recombinant c-Jun as substrate. Phosphorylation of the c-Jun can be analyzed by Western blot analysis using a phospho-c-Jun specific antibody. The kit specifically detects JNK, other kinase activity would not be detected.

II. Kit Contents:

Component	K431-40	K431-40	Color Code
	Part No.	40 assays	Cap Code
Kinase Extraction Buffer	K431-40-1	80 ml	NM
JNK Specific Antibody	K431-40-2	80 µl	Red
Protein A Sepharose	K431-40-3	2 ml	Clear
c-Jun Protein/ATP Mixture	K431-40-4	80 µl	Blue
Kinase Assay Buffer	K431-40-5	25 ml	WM
Phospho-cJun Specific Antibody	K431-40-6	50 µl	Green

III. General Consideration:

- Read the entire protocol before beginning the procedure.

IV. 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 being 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 (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. JNK Immunoprecipitation:

6. For each assay, add 2 µl JNK 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 vortexing 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 JNK Extraction Buffer and one time with 0.5 ml Kinase Assay Buffer.

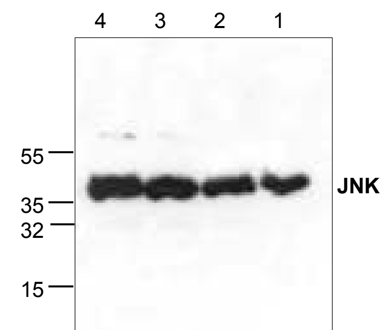
C. Kinase Assay:

10. Add 50 µl Kinase Assay Buffer to the washed Protein A beads, add 2 µl c-Jun Protein/ATP Mixture and incubate at 30°C for 1-4 hr.
11. Spin down the Protein A beads, and collect 30 µl of 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 any extra Protein A Beads in the sample.
13. Load the supernatant (20 µl) on 12% SDS-PAGE. Alternatively, the supernatant may be stored at -20°C for future use.

D. Western Immunoblotting:

14. 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.

FOR RESEARCH USE ONLY! Not to be used in humans.



Cat. #: K431-40
Western blot analysis of Phospho-c-Jun in various amounts of Jurkat cell lysate :
Lane 1: 50 µg
Lane 2: 100 µg
Lane 3: 200 µg
Lane 4: 400 µg

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