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ab273417
JNK Activity Assay Kit

View Kit datasheet: https://www.abcam.com/ab273417
(use https://www.abcam.cn/ab273417 for china, or
https://www.abcam.co.jp/ab273417 for Japan)

For the detection of JNK kinase activity in cell lysates using immunoprecipitated JNK to phosphorylate c-Jun which is subsequently detected by Western blot.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

JNK Activity Assay Kit (ab273417) 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.

2. Protocol Summary

Activate cells by desired method

Prepare cell lysate as directed

Determine the protein concentration of the lysate

Immunoprecipitate JNK

Perform kinase assay on the immunoprecipitate

Analyze by Western blot with phospho-specific c-Jun antibody

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Kinase Extraction Buffer	80mL	-20°C
JNK Antibody/JNK Specific Antibody	80µL	-20°C
Protein A Sepharose	2mL	-20°C
c-Jun Protein Mixture/c-Jun Protein/ATP Mixture	80µL	-20°C
Kinase Assay Buffer	25mL	-20°C
Phospho-cJun Antibody/Phospho-cJun Specific Antibody	50µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Centrifuge for lysate preparation
- Microfuge and tubes
- Equipment for SDS-PAGE and Western blotting
- PBS

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Hot plate/dry heat block or microplate incubator

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Reagents are supplied ready to use.

10. Sample Preparation

Preparation of cell lysate:

10.1 Activate cells by desired methods.
10.2 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 hour, before being harvested.
10.3 Wash Pellet cells (2-10 million/assay) once in 1X ice-cold PBS.
10.4 Lyse cells in 200 µL ice-cold JNK Extraction Buffer. Incubate on ice for 5 min.
10.5 Centrifuge pellet at 13,000 rpm for 10 minutes at 4°C. Transfer supernatant (Cell Lysate) to a new tube.
10.6 Perform assay protein concentration on the Cell Lysate. The Cell Lysate can be used immediately or freeze at -80°C for future use.

11. Assay Protocol

11.1 JNK Immunoprecipitation:

11.1.1 For each assay, add 2 µL JNK Antibody/JNK Specific Antibody (reacts with human, mouse, and rat) to 200 µL Cell Lysate (~50-400 µg total protein), and rotate for 45 minutes at room temperature.

11.1.2 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.

11.1.3 Centrifuge at 15,000 rpm for 2 min, remove supernatant.

11.1.4 Wash the protein A beads two times with 0.5 mL JNK Extraction Buffer and one time with 0.5 mL Kinase Assay Buffer.

11.2 Kinase Assay:

11.2.1 Add 50 µL Kinase Assay Buffer to the washed Protein A beads, add 2 µL c-Jun Protein Mixture/c-Jun Protein/ATP Mixture and incubate at 30°C for 1-4 hr.

11.2.2 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).

11.2.3 Boil the samples for 3 min. Microcentrifuge for 2 minutes to spin down any extra Protein A Beads in the sample.

11.2.4 Load the supernatant (20 µL) on 12% SDS-PAGE. Alternatively, the supernatant may be stored at -20°C for future use.

11.3 Western Immunoblot:

11.3.1 Perform Western blot analysis using the rabbit anti-Phospho-cJun (Ser 73) Specific Antibody at 1/1000 dilution.

11.3.2 A 35 kDa band corresponding to the phosphorylated cJun protein should be detected in JNK activated samples.

12. Typical Data

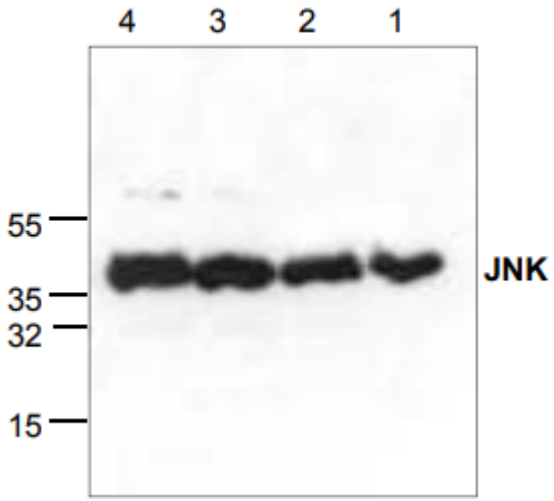

Figure1: Western blot analysis of Phospho-c-Jun in various amounts of Jurkat cell lysate. Lane 1: 50 µg Lane 2: 100 µg Lane 3: 200 µg Lane 4: 400 µg.

13. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

14. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)