

# Affinity-purified Rabbit Anti-human/mouse/rat pan JNK Antibody

## ORDERING INFORMATION

**Catalog Number:** AF1387

**Lot Number:** ICZ09

**Size:** 100 µg (sufficient for 500 mL of blotting solution)

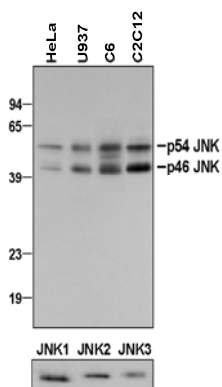
**Storage:** -20° C

**Specificity:** human, mouse and rat JNK1, JNK2 and JNK3

**Immunogen:** *E. coli*-derived recombinant human JNK1

**Ig Type:** rabbit IgG

**Applications:** Western blot  
Immunohistochemistry



### Detection of JNK isoforms with AF1387.

Lysates from human HeLa and U937 cells, rat C6 cells, and mouse C2C12 cells were resolved by SDS-PAGE (upper panel), as were 1 ng amounts of recombinant JNK1, JNK2 and JNK3 (lower panel). Following electrophoresis, lysates and recombinant protein were transferred to Immobilon membranes and immunoblotted with 0.2 µg/mL rabbit anti-pan JNK, as described in *Protocols for Immunoblotting*. Three minute (upper) and 1 minute (lower) exposures to film are shown.

## Preparation

Rabbit antibodies were raised against purified, *E. coli*-derived recombinant human c-Jun N-terminal kinase-1 (JNK1), also known as stress-activated protein kinase-1 (SAPK1) and mitogen-activated protein kinase-8 (MAPK8) (GenBank accession # NM\_002750). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified on a protein A column.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute in 100 µL of PBS containing 0.02% NaN<sub>3</sub>.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

## Specificity

The antibody detects endogenous human, mouse and rat p46 and p54 JNK, the isoforms of JNK most frequently detected by Western blot. The antibody detects recombinant JNK1, JNK2 and JNK3.

## Applications

**Western blot** - An antibody concentration of 0.2 µg/mL is recommended.

### Protocols for Immunoblotting

#### Blotting Buffer

25 mM Tris, pH 7.4

0.5 M NaCl

0.1% Tween 20®

#### Blocking Solution

5% nonfat dry milk in

blotting buffer

Adjust pH to 7.4

#### Antibody Solution

5% nonfat dry milk

in blotting buffer

Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.2 µg/mL rabbit anti-human/mouse/rat pan JNK.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-rabbit IgG (Amersham).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

**Cell lysates for Western blottings** - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2 x 10<sup>6</sup> - 1 x 10<sup>7</sup> cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

**Immunohistochemistry** - This antibody will detect JNK in cells and tissues. The working dilution is 5 - 15 µg/mL. For chromogenic detection of labeling, use R&D Systems Cell and Tissue Staining Kits (CTS Series).

**Optimal dilutions should be determined by the individual laboratory.**