

ORDERING INFORMATION

Catalog Number: MAB1604

Clone: 304328

Lot Number: VYD01

Size: 100 µg (sufficient for 200 mL of blotting solution)

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Specificity: human/mouse/rat MKK6

Immunogen: *E. coli*-derived rhMKK6

Ig class: rat IgG_{2b}

Recommended Applications:

Western blot
Immunohistochemistry

Background

Mitogen-activated protein kinase kinase 6 (MKK6), also known as MEK6 and MAP2K6, is activated by proinflammatory cytokines and environmental stresses. Activation occurs through phosphorylation by several upstream MAPK kinase kinases, including ASK1 and TAK1. Both MKK6 and the closely related MKK3 are dual specificity protein kinases, phosphorylating and activating the p38 MAP kinases at T and Y positions within the phosphoacceptor sequence T-G-Y.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived recombinant human MKK6 (rhMKK6 isoform 1; aa 1 - 334; Accession # P52564). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

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

Reconstitution

Reconstitute in PBS containing 0.02% NaN₃.

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

This antibody was selected for its ability to detect endogenous 38 kDa MKK6 in Western blots. In Western blots and direct ELISAs, this antibody does not cross-react with rhMKK3.

Applications

Western Blot - An antibody concentration of 0.5 µg/mL is recommended.

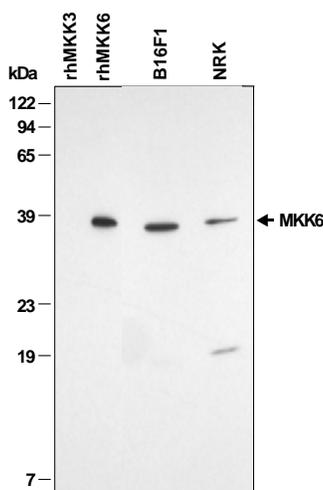
Immunohistochemistry - This antibody was used at a concentration of 25 µg/mL with appropriate secondary reagents to detect MKK6 in paraformaldehyde-fixed human PBMC. For chromogenic detection of labeling, the use of R&D Systems' Cell and Tissue Staining Kits (CTS Series) is recommended.

Protocols for Immunoblotting:

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	5% nonfat dry milk in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween® 20		

- Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
- Incubate the membrane overnight at 4° C in Antibody Solution containing 0.5 µg/mL anti-human/mouse/rat MKK6.
- Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
- Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated goat anti-rat IgG (Zymed).
- Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
- Detect with WesternGlo Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) or equivalent.

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 2×10^6 - 1×10^7 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.



Detection of MKK6 with MAB1604.

Recombinant human (rh) MKK3 (2 ng), rhMKK6 (2 ng), and lysates of mouse B16F1 and rat NRK cells were resolved by SDS-PAGE. Following electrophoresis, recombinant proteins and lysates were transferred to an Immobilon-P membrane and immunoblotted with 0.5 µg/mL anti-MKK6, as described in *Protocols for Immunoblotting*. A twenty second exposure to film is shown.

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