

Affinity-Purified Goat Anti-human MKK7 Antibody

ORDERING INFORMATION

Catalog Number: AF3579

Lot Number: YQA01

Size: 100 µg (sufficient for 50 mL of blotting solution)

Storage: -20° C

Specificity: human MKK7

Immunogen: *E. coli*-derived rhMKK7

Ig Type: goat IgG

Applications: Western blot
Immunohistochemistry

Background

Mitogen-activated protein kinase kinase 7 (MKK7 or MAP2K7), also known as MEK7 and Jun kinase kinase 2 (JNKK2), is activated by proinflammatory cytokines and environmental stresses. Activation occurs through phosphorylation at Ser271 and Thr275 by several upstream MAPK kinase kinases (MAP3Ks). MKK7 is a dual specificity protein kinase, phosphorylating and activating the JNK family of MAP kinases at Thr and Tyr positions within the phosphoacceptor sequence Thr-Pro-Tyr.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human MKK7 (rhMKK7; aa 1 - 419; Accession # O14733). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

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 detects endogenous human MKK7 at 47 kDa using Western blot. The antibody does not cross-react with other recombinant human mitogen-activated protein kinase kinases.

Applications

Western blot - An antibody concentration of 2.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween 20®

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

Antibody Solution

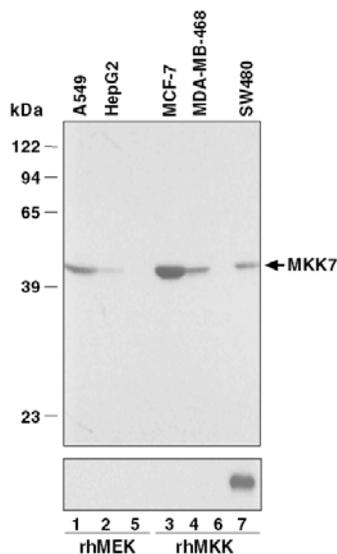
5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P 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 2.0 µg/mL goat anti-human MKK7.
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 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent Detection Reagent (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.

Immunohistochemistry - This antibody was used at a concentration of 3 - 10 µg/mL with the appropriate secondary reagents to detect MKK7 in paraffin-embedded sections of normal human tonsil and placenta. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by the individual laboratory.



Detection of MKK7 with AF3579. 5 ng of rhMEKs and rhMKKs (lower panel), and lysates from human A549, HepG2, MCF-7, MDA-MB-468, and SW480 cells (upper panel) were resolved by SDS-PAGE. Following electrophoresis, proteins and lysates were transferred to an Immobilon-P membrane and immunoblotted with 2.0 µg/mL anti-MKK7, as described in *Protocols for Immunoblotting*. Three minute (upper) and two hour (lower) exposures to film are shown.