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AM26673AF-N Monoclonal Antibody to MAP2K1 - Azide Free

Alternate names: Dual specificity mitogen-activated protein kinase kinase 1, ERK activator kinase 1, MAP kinase kinase 1, MAPK/ERK kinase 1, MAPKK 1, MEK1, PRKMK1

Quantity: 0.1 ml

Concentration: 1.0 mg/ml

Background: The MAP kinases mediate a wide range of cellular functions via a variety of signal transduction pathways in eukaryotic cells. The most studied mammalian MAP kinase pathway is the Ras-Raf-MEK-ERK pathway. The binding of GTP to Ras protein initiates a phosphorylation cascade through Raf-1 and MEK1/2 (MAPK kinase), which results in stimulation of the MAP kinases, ERK1/2. Upon stimulation, ERKs are known to phosphorylate a variety of cytosolic substrates and are also translocated into the nucleus where they initiate the transcription of immediate early genes. The Ras-Raf-MEK-ERK pathway is stimulated by various growth factors and extracellular stimuli and plays important roles in cell survival, differentiation and proliferation. The observation that MEK1/2 inhibitors potentiate the antitumor activity of various cytotoxic agents, including ara-C, cisplatin and paclitaxel, suggests a possible role for MEK1/2 inhibitors in the treatment of human malignancies.

Uniprot ID: Q02750

NCBI: NP_002746.1

GeneID: 5604

Host / Isotype: Mouse / IgG2a

Clone: 4A5

Immunogen: Recombinant Xenopus MAPKK

Format:
State: Liquid Ig fraction without preservatives
Purification: Protein A Agarose
Buffer System: PBS containing 50% glycerol
Preservatives: None

Applications:
Western blot: 1-5 µg/mL for chemiluminescence detection system.
Immunoprecipitation: 5-10 µg/200 µL of cell extract from 5x10e6 cells.
For details see protocol below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with MAPKK (MEK1).

Species Reactivity: **Tested:** Human, mouse, rat, xenopus

Add. Information: This product was originally produced by MBL International.

Storage: Upon receipt, store undiluted (in aliquots) at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

1. Miura, K., et al., J. Immunol. 167, 7027-7037 (2001).
2. Miura, K., et al., Blood 96, 2199-2205 (2000).
3. Fukuda, M., et al., J. Biol. Chem. 271, 20024-20028 (1996).
4. Fukuda, M., et al., J. Biol. Chem. 269, 33097-33101 (1994).
5. Kosako, H., et al., EMBO J. 13, 2131-2138 (1994).
6. Otsu, M., et al., FEBS Lett. 320, 246-250 (1993).
7. Kosako, H., et al., EMBO J. 12, 787-794 (1993).

Protocols:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary. (Positive controls for Western blotting; Jurkat, Raji, HeLa, NIH/3T3, PC12)

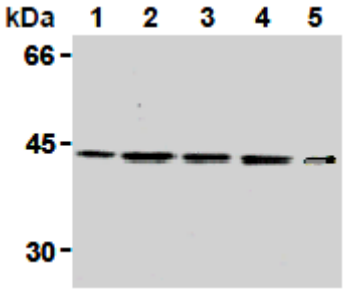
Immunoprecipitation

1) Collect the cultured cells from 75-cm2 flask (containing about 0.5-1 x 10e7 cells).
2) Wash the cells 2 times with PBS and suspend with 1,200 µL of cold Lysis buffer (50 mM Tris-HCl pH 7.4, 250mM NaCl, 0.1% NP-40, 2mM EDTA) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 15 minutes, then sonicate briefly (up to 10 seconds).
3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.

4) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4oC. Supernatant is equally divided into another two tube.
6) Add primary antibody as suggested in the APPLICATIONS into the supernatant. Vortex briefly and incubate with gently agitation for 60-120 minutes at 4°C.
7) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4oC.
8) Wash the beads 3-5 times with ice-cold IP buffer (10 mM Tris-HCl, 250 mM NaCl, 0.1% NP-40, pH7.4) (centrifuge the tube at 2,500 x g for 10 seconds).
9) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Load 15 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
14) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG kappa chain diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
15) Wash the membrane with PBS-T (10 minutes x 3 times).
16) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
17) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose to an X-ray film in a dark room for 3 minutes.
19) Develop the film as usual. The condition for exposure and development may vary. (Positive control for Immunoprecipitation; Jurkat)

Pictures:

Western blot analysis of MAPKK (MEK1) expression in Jurkat (1), Raji (2), HeLa (3), NIH/3T3 (4) and PC12 (5) using AM26673AF-N.



MEK1Immunoprecipitation of MAPKK/MEK1 from Jurkat with mouse IgG2a (1) or AM26673AF-N (2). After immunoprecipitatedwith the antibody, immunocomplexwas resolved on SDS-PAGE and immunoblottedwith AM26673AF-N. Jurkat crude lysatewas resolved in lane 3.3020