Monoclonal Anti-human/mouse/rat MEK2 Antibody

ORDERING INFORMATION

Catalog Number: MAB2855

Clone: 300317

Lot Number: VXV01

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

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

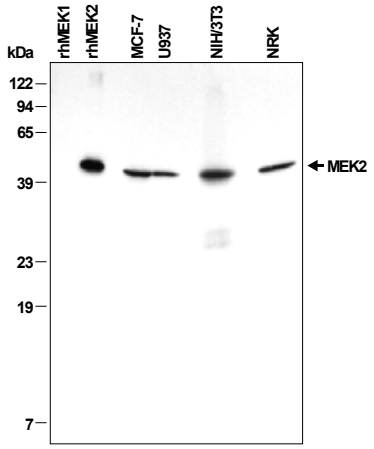
Storage: -20° C

Specificity: human/mouse/rat MEK2

Immunogen: *E. coli*-derived rhMEK2

Ig class: mouse IgG_{2A}

Recommended Applications:
Western blot
Immunohistochemistry



Detection of MEK2 with MAB2855. Recombinant human (rh) MEK1 (5 ng), rhMEK2 (5 ng) and lysates of human MCF-7, human U937, mouse NIH/3T3, 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-MEK2, as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.

Background
Mitogen-activated protein kinase kinase 2 (MEK2 or MAP2K2), also known as MKK2, belongs to the STE family of kinases. Both MEK2 and the related MEK1 are dual-specificity kinases, phosphorylating and activating the mitogen-activated protein kinases ERK1 and ERK2 at T and Y positions within the phosphoacceptor sequence T-E-Y. Activation of MEK2 by Raf occurs through phosphorylation at S222 and S226.

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived full-length recombinant human MEK2 (rhMEK2; aa 1 - 400; Accession # P36507). The IgG fraction of the tissue culture supernatant was purified by protein G 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% NaN_3.

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 MEK2 at 45 kDa in Western blots and immunohistochemistry experiments. In direct ELISAs, this antibody does not cross-react with rhMEK1 or rhMEK5.

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 MEK2 in human peripheral blood mononuclear cells. 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	2% nonfat dry milk in	2% nonfat dry milk in
0.15 M NaCl	Blotting Buffer	Blotting Buffer
0.1% Tween 20	Adjust pH to 7.4	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 0.5 μg/mL anti-human/mouse/rat MEK2.
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 goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. 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 x 10^6 - 1 x 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. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Optimal dilutions should be determined by the individual laboratory.

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