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# Product Information

**Monoclonal Anti-MAP Kinase 2 (ERK-2), clone 1B3B9**
produced in mouse, purified immunoglobulin

Catalog Number **M7431**

## Product Description

Monoclonal Anti-MAP Kinase 2 (MAPK2 or Extracellular Regulated Protein Kinase, ERK-2) (mouse IgG2a isotype) is produced using HPLC purified recombinant mouse MAPK2 as immunogen. The IgG2a antibody is derived from the B9 hybridoma produced by the fusion of mouse myeloma cells (SP2/Ag14) and splenocytes from an immunized Balb/c mouse. The antibody is purified from ascites fluid using protein G chromatography.

Anti-MAP Kinase 2 recognizes the 42 kDa MAPK2 encoded by the *mapk* gene. The antibody may weakly react with MAPK1 (ERK1) and only binds denatured MAP kinase. It reacts with mouse, human, and rat MAPK2.

Anti-MAP Kinase 2 may be used for the detection of MAPK2 by immunoblotting RIPA cell lysates of human A431 carcinoma cells, mouse 3T3 fibroblasts or rat L6 skeletal fibroblasts. Anti-MAP Kinase 2 may also be used for immunoprecipitation of MAPK2 from a RIPA lysate from mouse 3T3 fibroblasts.

MAPK2 or ERK-2 is a part of complicated signal transduction cascade. This cascade can be initiated by growth factors binding to receptor tyrosine kinases, by the activation of low molecular weight GTP-binding proteins or by G protein-coupled receptors. The initiation of this pathway has been linked to changes in several cellular pathways, including proliferation, differentiation, cellular morphology and oncogenesis. The pathway begins with the activation of a MAP kinase kinase kinase, such as Raf and MEKK, that subsequently activates a MAP kinase kinase, such as MEK1 or MEK2. MEK then phosphorylates both tyrosine and threonine residues resulting in activation of a MAP kinase,[1, 2] such as ERK 1($p44^{mapk}$)[3] or ERK 2($p42^{mapk}$).[4]

Phosphorylation at both the tyrosine and threonine residues is necessary for full enzymatic activity.[5] Following activation, MAP kinase phosphorylates several nuclear targets, including transcription factors. In addition, MAP kinase phosphorylates membrane proteins and cytoskeletal proteins.[6, 7] Termination of MAP kinase signaling appears to be mediated by MAP kinase phosphatase, MKP-1, a dual specificity Thr/Tyr phosphatase which dephosphorylates and inactivates MAP kinase.[8] MAP kinases are widely expressed in the central nervous system, thymus, spleen, heart, lung and kidney, and are expressed in high levels in PC-12 cells and in fibroblasts.[2, 6] Antibodies that react specifically with MAP kinase may be used to study the specific activation requirements, differential tissue expression and intracellular localization of MAP kinase in normal and neoplastic tissue.

## Reagent

Supplied in phosphate buffered saline containing 0.05% sodium azide.

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Store at –20 °C. Aliquot to avoid repeated freezing and thawing. Do not store in frost-free freezers. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

## Product Profile

Immunoblotting: a minimum working concentration of 0.5 μg/ml is recommended for cell lysates using enhanced chemiluminescence.

Immunoprecipitation: 4 μg will immunoprecipitate MAPK 2 from 0.5-1 mg of a mouse 3T3 fibroblast lysate, which has been boiled and cooled to denature MAPK2. The antibody will only bind denatured MAPK2

**Note**: In order to obtain best results and assay sensitivity in different techniques and preparations, we recommend determining optimal working dilutions by titration test.

**Procedure**

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 µg/µL total cell protein in a microcentrifuge tube with PBS, Catalog Number P3813.
2. Add 4µg of Anti-MAP Kinase 2 to 0.5-1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µL packed beads), Catalog No. P2545.
5. Gently rock reaction mixture at 4 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x *g*), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µL 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. The beads are pelleted by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM $Na_3VO_4$, and 1 mM NaF.

**References**


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