Monoclonal Anti-human/mouse/rat ERK4 Antibody

ORDERING INFORMATION

Catalog Number: MAB3914

Clone: 355810

Lot Number: YRF01

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

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

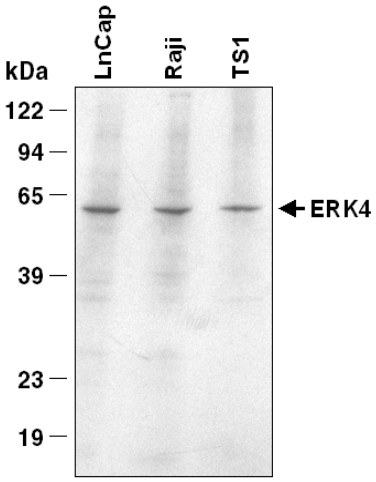
Storage: -20° C

Specificity: human/mouse/rat ERK4

Immunogen: *E. coli*-derived rhERK4 (aa 1 - 126)

Ig class: rat IgG_{2A}

Recommended Application:
Western blot
Immunocytochemistry



Detection of ERK4 with MAB3914. Lysates from human LnCap, human Raji, and mouse TS1 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 2 μg/mL anti-ERK4, as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.

Background

Extracellular signal-regulated kinase 4 (ERK4) is a serine/threonine protein kinase also known as mitogen-activated protein kinase 4 (MAPK4), p63MAPK, and ERK3-related kinase. ERK4 and the structurally similar ERK3 (MAPK6) lack the phosphoacceptor sequence T-X-Y present in other MAPKs. ERK4 complexes with ERK3 to activate MAPK-activated protein kinase 5 (MK5), also known as PRAK.

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 ERK4 (rhERK4; aa 1 - 126; Accession # P31152). 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 with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 μg/mL.

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

The antibody detects endogenous human, mouse, and rat ERK4 at 63 kDa using Western blot.

Application

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

Optimal dilutions should be determined by each laboratory for each application.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	2% nonfat dry milk in Blotting Buffer	2% nonfat dry milk in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween® 20		

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 rat anti-human/mouse/rat ERK4.
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 1:1000 dilution of HRP-conjugated goat anti-rat IgG (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent detection reagents (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.

Immunocytochemistry - This antibody was used at a concentration of 8 - 25 μg/mL with appropriate secondary reagents to detect ERK4 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.

R&D Systems, Inc.
1-800-343-7475

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