Biotinylated Affinity-Purified Goat Anti-human ERK1 Antibody

ORDERING INFORMATION

Catalog Number: BAF1879

Lot Number: KAT01

Size: 50 μg (sufficient for 250 mL of blotting solution)

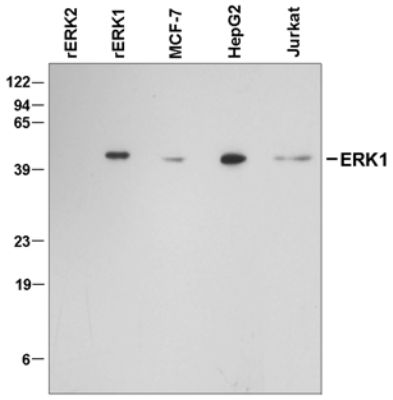
Storage: -20° C

Specificity: human ERK1

Immunogen: *E. coli*-derived recombinant human ERK1

Ig Type: goat IgG

Application: Western blot



Detection of ERK1 with BAF1879. Lysates from human MCF-7, HepG2, and Jurkat cells, as well as 2 ng of recombinant human ERK1 and ERK2 (with polyHis tags), were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.2 μg/mL biotinylated goat anti-ERK1, as described in *Protocols for Immunoblotting*. A 3 minute exposure to film is shown.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human extracellular signal-regulated kinase-1 (ERK1), also known as mitogen-activated protein kinase-3 (MAPK3) and p44 MAPK (Accession # XM_055766). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein, further purified by isolating the IgG fraction, and biotinylated.

Formulation

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) containing 50 μg of bovine serum albumin per 1 μg of antibody.

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

The antibody detects endogenous human ERK1. The antibody does not detect recombinant or endogenous human ERK2 using Western blots.

Application

Western blot - an antibody concentration of 0.2 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	5% 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 0.2 μg/mL biotinylated goat anti-human ERK1.
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 25 ng/mL HRP-conjugated streptavidin (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham) 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.

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