

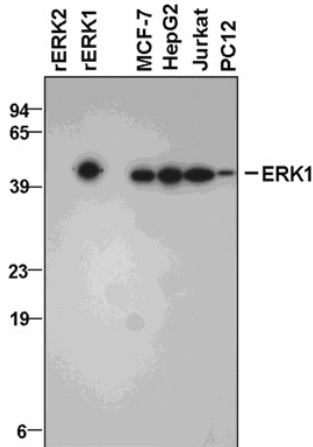
ERK1

Data Sheet

Catalog Number:	RA15000	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Rat, Mouse, Human
Immunogen Sequence:	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).	Format:	Liquid 1mg/ml phosphate-buffered saline with 5% trehalose
Applications:	Western blotting: 0.1 ug/ml Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. 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. <i>Avoid repeated freeze-thaw cycles.</i>		

Western blotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4 0.15 M NaCl 0.1% Tween 20	5% nonfat dry milk in Blotting Buffer, Adjust pH to 7.4	5% nonfat dry milk in Blotting Buffer Adjust pH to 7.4



Detection of ERK1 with RA15000

2 ng of recombinant ERK1 and ERK2; lysates from human MCF-7, HepG2, Jurkat and rat PC12 cells resolved by SDS-PAGE, transferred to Immobilon membrane and immunoblotted with 0.1 µg/mL rabbit anti-ERK1

1. Transfer the electrophoresed proteins to Immobilon 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 0.1µg/mL rabbit anti-human/mouse/rat 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 a HRP-conjugated anti-rabbit IgG secondary antibody.
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

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 2×10^6 - 1×10^7 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.

Immunohistochemistry

This antibody will detect ERK2 in cells and tissues. The working dilution is 5 - 15 µg/mL.

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