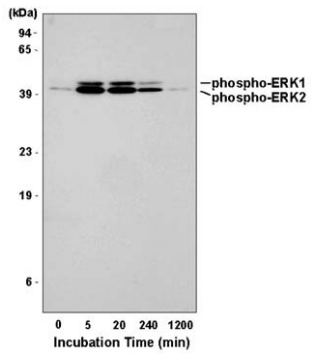
NEUROMICS

phosphoERK1/2 Data Sheet

Catalog Number:	RA15002	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Rat, Mouse, Human
Immunogen Sequence:	synthetic phosphopeptide including residue T202/Y204 of extracellular signal-regulated kinase-1 (ERK1; MAPK3, p44 MAPK) and residues T185/Y187 of ERK2 (MAPK1, p42 MAPK)	**Format:**	Liquid 1mg/ml phosphate-buffered saline with 5% trehalose
Applications:	**Western blotting: 0.1 ug/ml** **Immunohistochemistry: 5-15 ug/ml** Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator. Recognizes ERK1 and ERK2 dually phosphorylated at T202/Y204 and T185/Y187, respectively.		
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. *Avoid repeated freeze-thaw cycles.*		

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 and ERK2 phosphorylated at T202/Y204 and T185/Y187, respectively. Human HeLa cells were incubated with 200 nM PMA for the indicated times. Total cell lysates in gel sample buffer were resolved by SDS-PAGE, transferred to Immobilon membrane and immunoblotted with 0.1 µg/mL anti-phosphERK1/2.

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 phosphoERK1/2.
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-goat 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 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.

Immunohistochemistry

This antibody will detect phosphoERK1/2 in cells and tissues. The working dilution is 5 - 15 µg/mL.

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