Rabbit (polyclonal) Anti-ERK1&2 [pTpY$^{185/187}$] Phosphorylation Site Specific Antibody Alexa Fluor® 488 Conjugate

PRODUCT ANALYSIS SHEET

Catalog Number: 44680A1 (50 Tests)

Lot Number: See product label

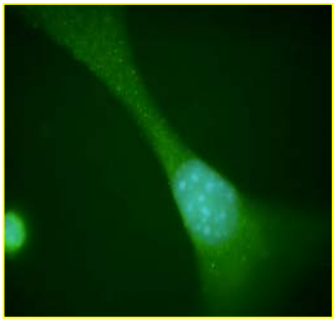
Volume: 100 µL

Suggested Dilutions: The optimal dilution should be determined empirically for each cell type and stimulation protocol. The recommended starting dilution for most experimental systems is 1:50.

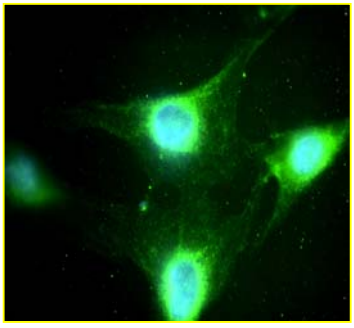
Formulation: Alexa Fluor® 488-conjugated purified immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), with 0.2% BSA and 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.).

Target Summary: ERK (extracellular signal-regulated kinase), also known as MAPK (mitogen-activated protein kinase) has two closely related isoforms of 44 kDa and 42 kDa, respectively. These kinases belong to a family of serine/threonine kinases that are activated upon treatment of cells with a large variety of stimuli including mitogens, hormones, growth factors, cytokines, and bioactive peptides. Cell stimulation induces the activation of a signaling cascade, the downstream effects of which have been linked to the regulation of cell growth, differentiation and the cytoskeleton. ERK1 and ERK2 are fully activated by phosphorylation within a Thr-Glu-Tyr motif of the activation loop on both a threonine and a tyrosine residue by MEKs (MAPK/ERK kinases), thereby greatly elevating the activity of the ERK1&2 enzymes.

Reactivity: Human, mouse, and rat ERK1&2.



A. NIH3T3 (Untreated)



B. NIH3T3+PDGF

Immunofluorescence Staining:
NIH3T3 cells were serum starved and either left untreated (A) or treated with PDGF (50 ng/mL for 15 minutes) (B). Cells were fixed prior to immunostaining with the anti-ERK1/2 [pTpY$^{185/187}$] Alexa Fluor® 488 conjugate. The data show the upregulation of ERK1/2 [pTpY$^{185/187}$] in the PDGF-treated cells, as indicated by the enhanced Alexa Fluor® 488 signal in these cells.

Please visit our website (invitrogen.com) to view the images in full color.

Production: This antibody was produced against a chemically synthesized phosphopeptide derived from the region of ERK1 that contains threonine 202 (corresponding to 185 in ERK2) and tyrosine 204 (corresponding to 187 in ERK2). The antibody was affinity purified by sequential epitope-specific chromatography, then conjugated to Alexa Fluor® 488 under optimal conditions.

Storage: Store at 2-8°C for up to one month. For long term storage, apportion the antibody into working aliquots and store at –20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody. Protect from light.

Expiration Date: Expires one year from the date of receipt when stored as instructed.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

Related Products:

Antibodies:

ERK1/2 [pTpY$^{185/187}$], Cat. # 44680G	JNK1, Cat. # 44690G
MEK1/2 [pS^{222}], Cat. # 44452	Alexa 488 PRAS40 [pT^{246}], Cat. # 441100A1
JNK1/2 [pTpY$^{183/185}$], Cat. # 44680G	Alexa 488 Pyk2 [pY^{402}], Cat. # 44618A1
p38 MAPK [pTpY$^{180/182}$], Cat. # 44684G	Alexa 488 Paxillin [pY^{118}], Cat. # 44722A1
ERK1/2, Cat. # 44654G	Alexa 488 Src [pY^{418}], Cat. # 44660A1

References:

Karasewski, L. and A. Ferreira (2003) MAPK signal transduction pathway mediates agrin effects on neurite elongation in cultured hippocampal neurons. J. Neurobiol. 55(1):14-24.

Janne, P.A., et al. (2002) Inhibition of epidermal growth factor receptor signaling in malignant pleural mesothelioma. Cancer Res. 62(18):5242-5247.

Robinson, F.L., et al. (2002) Identification of novel point mutations in ERK2 that selectively disrupt binding to MEK1. J. Biol. Chem. 277(17):14844-14852.

Grammatikakis, N., et al. (2002) The role of Hsp90N, a new member of the Hsp90 family, in signal transduction and neoplastic transformation. J. Biol. Chem. 277(10):8312-8320.

Janulis, M., et al. (2001) A novel mitogen-activated protein kinase is responsive to Raf and mediates growth factor specificity. Mol. Cell. Biol. 21(6):2235-2247.

Nanki, T., et al. (2001) Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J. Immunol. 167(9):5381-5385.

Tong, T., et al. (2001) Involvement of the MAP kinase pathways in induction of GADD45 following UV radiation. Exp. Cell Res. 269(1):64-72.

Cobb, M.H. (1999) MAP kinase pathways. Prog. Biophys. Mol. Biol. 71(3-4):479-500.

Fanger, G.R. (1999) Regulation of the MAPK family members: role of subcellular localization and architectural organization. Histol. Histopathol. 14(3):887-894.

Xu, S., et al. (1997) Differential regulation of mitogen-activated protein/ERK kinase (MEK)1 and MEK2 and activation by a Ras-independent mechanism. Mol. Endocrinol. 11(11):1618-1625.

Immunofluorescence Staining of Adherent Cells:

BioSource's phosphorylation site specific antibodies have been used successfully in many antibody-based techniques, including Western blot analyses and sandwich immunoassays (i.e., phosphoELISA™ and Luminex™ assays). When used in immunohistochemistry, these antibodies not only detect phosphorylation events, but also provide valuable information about the subcellular localization of phosphorylated proteins. BioSource's Alexa Fluor® 488-conjugated phosphorylation site specific antibodies have been developed specifically to provide a facile method for detecting protein phosphorylation and localization by immunohistochemistry.

Procedure:

1. Plate adherent cells onto glass cover slips.
2. Culture the cells for 16 hours in appropriate medium. It is important to note that serum starvation may be necessary in some stimulation procedures.
3. Stimulate the cells as desired.
4. Remove the medium from the cells by decanting.
5. Fix the cells by pipetting 200 µL 95% ice cold methanol onto the slides. (Fixatives composed of equal volumes of acetone and methanol, or 4% paraformaldehyde, have also been used successfully.)
6. Incubate for 10 minutes at -20°C.
7. Remove the fixative solution by decanting.
8. Pipette 200 µL Blocking Buffer onto the slides. (Blocking Buffer Formulation: 3% BSA/TBST/0.1% Triton X-100, supplemented with protease inhibitor cocktail [Sigma Cat. # P8340] and phosphatase inhibitor cocktail I and II [Sigma Cat. # P2850 and P5726]).
9. Incubate for 30 minutes at room temperature.
10. Remove the Blocking Buffer by decanting.
11. Pipette 100-200 µL Alexa Fluor® 488 conjugated phosphorylation site specific antibody, diluted in Blocking Buffer, onto the cover slip.
12. Incubate for two hours at room temperature or overnight at 2-8°C.
13. Remove the antibody solution by decanting.
14. Wash the cells twice with phosphate buffered saline, pH 7.2, 10 minutes each.
15. Add one drop of Vectashield solution (Vector Lab, H-1500) to prevent photobleaching the fluorescent signal. Vectashield solution contains DAPI to counterstain the nucleus. Mount the cover slip on a microscopic slide.
16. Examine the slides with an immunofluorescence microscope (e.g., Zeiss Axioplan 2). We suggest using the 100x oil immersion lens.