Rabbit (polyclonal) Anti-ERK1&2 pan Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-654G
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by peptide affinity chromatography.
Immunogen:	The antibody was produced using a synthetic peptide derived from amino acids 317-339 within the carboxyl-terminal half of the human ERK1 protein. The sequence is conserved in human and rat.
Target Group:	ERK (extracellular signal regulated kinase), also known as MAPK (mitogen-activated protein kinase) has two closely related isoforms designated ERK1 and ERK2 (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 and differentiation as well as the cytoskeleton. ERK1 and ERK2 are phosphorylated within the activation loop on both a threonine and a tyrosine residue (within a Thr-Glu-Tyr motif) by MEKs (MAPK/ERK kinases), thereby greatly elevating the activity of ERK1&2.
Cross-Reactivity:	Human, mouse and rat ERK1&2. Other species have not been tested.
Applications:	This antibody has been used in Western blotting.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Controls Used:	Human epidermoid carcinoma A431, mouse NIH3T3 and rat pheochromocytoma (PC12) cells.
Related Products:	**Antibodies:** ERK1&2 [$pTpY^{185/187}$], Cat. # 44-680G JNK1&2 [$pTpY^{183/185}$], Cat. # 44-682G p38 [$pTpY^{180/182}$], Cat. # 44-684G ERK5 [$pTpY^{218/220}$], Cat. # 44-612G ERK5, Cat. # 44-688G **Extracts:** PC12 cell extracts +/- Sorbitol, Cat. # 55-170

This product is for research use only. Not for use in diagnostic procedures.

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

PI44654G (Rev 11/08) DCC-08-1089

References:

Wang, X., et al. (2004) Interactions between extracelular signal-regulated protein kinase 1, 14-3-3ε, and heat shock factor 1 during stress. J. Biol. Chem. 279(47):49460-49469 (cites the use of cat. # 44-680G).

Tanimura, S., et al. (2002) Prolonged nuclear retention of activated extracellular signal-regulated kinase 1/2 is required for hepatocyte growth factor-induced cell motility. J. Biol. Chem. 277(31):28256-28264.

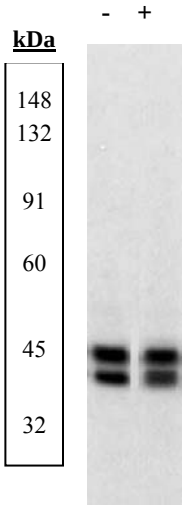
Janulis, M., et al. (2001) A novel mitogen-activated protein kinase is responsive to Raf and mediates growth factor specificity. Mol. Cell. Biol. 21(6):2235-2247 (cites the use of cat. # 44-612G, 44-654G, 44-680G, 44-682G, 44-684G, 44-688G and 44-690G).

Sweatt, J.D. (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J. Neurochem. 76(1):1-10 (cites the use of this antibody).

Widmann, C., et al. (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. 79(1):143-180.

Khokhlatchev, A., et al. (1997) Reconstitution of mitogen-activated protein kinase phosphorylation cascades in bacteria. Efficient synthesis of active protein kinases. J. Biol. Chem. 272(17):11057-11062.

Boulton, T.G. and M.H. Cobb (1991) Identification of multiple extracellular signal-regulated kinases (ERKs) with antipeptide antibodies. Cell. Regul. 2(5):357-371.



Western Blot

Extracts of PC12 cells unstimulated (-) or stimulated with 20 ng/mL NGF for 20 minutes (+) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. The membrane was blocked with a 5% BSA-TBST buffer overnight at 4°C, then incubated with 0.5 µg/mL ERK1&2 antibody for two hours at room temperature in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase (Cat. # ALI4405) and signals were detected using the Tropix WesternStar™ method.

The data show that the ERK1&2 antibody allows the total amount of ERK1&2 to be measured (non-phoshphorylated as well as phosphorylated). The resulting signals serve as useful controls in assessing the degree of up-regulation as detected using an antibody to the dually phosphorylated (active) form of ERK1&2 (44-680G).

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of nitrocellulose membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, PVDF may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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