Rabbit (polyclonal) Anti-ERK5/BMK1 pan, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44-688G

Lot Number: See product label

Quantity/Volume: See product label

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 1.0mg/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 epitope-specific affinity chromatography.

Immunogen: The antiserum was produced against a chemically synthesized peptide derived from residues 334-349 of the human ERK5/BMK1 protein. Full-length ERK5/BMK1 has a Mr of ~88 kDa, however, truncated versions of ~30-35 kDa have also been observed.

Specificity: The antibody recognizes the extracellular signal-related protein kinase/big MAP kinase 1 (ERK5/BMK1) enzyme independent of its phosphorylation state. Validation included recognition of full-length and truncated recombinant versions of ERK5/BMK1 when expressed in HEK 293 (human embryonal kidney) cells. Due to the generally low abundance of endogenous ERK5 observed with many cell types, overexpression or immunoprecipitation of ERK5 protein may be required to provide robust signals.

Reactivity: This antibody cross-reacts with mouse (Karihaloo et al., 2001).

Applications: The antibody has been used in Western blotting.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at 0.1-1.0 µg/mL. The optimal antibody concentration should be determined empirically for each specific application.

Storage: Store at –20ºC. 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: HEK293 cells transiently co-transfected with plasmids expressing ERK5 kinase domain (ERK5kin) and constitutively activated MEK5 (MEK5D-D).

Related Products:

Antibodies:

ERK5 [pYpY$^{218/220}$], Cat. # 44-612G

ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G

JNK1&2 [pTpY$^{183/185}$], Cat. # 44-682G

p38 [pTpY$^{180/182}$], Cat. # 44-684G

ERK1&2, Cat. # 44-654G

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References:

Garaude J, et al. (2005) The role of ERK5 in T-cell signalling. Scand. J. Immunol. 62(6):515-520.

Wang X. and C. Tournier (2005) Regulation of cellular functions by the ERK5 signalling pathway. Cell Signal. [Epub ahead of print].

Buschbeck, M., et al. (2002) Phosphotyrosine-specific phosphatase PTP-SL regulates the ERK5 signaling pathway. J. Biol. Chem. 277(33):29503-29509 (cites the use of cat. # 44-612G).

Shao, Y., et al. (2002) Pincher, a pinocytic chaperone for nerve growth factor/TrkA signaling endosomes. J. Cell. Biol. 157(4):679-691 (cites the use of cat. # 44-612G in immunostaining).

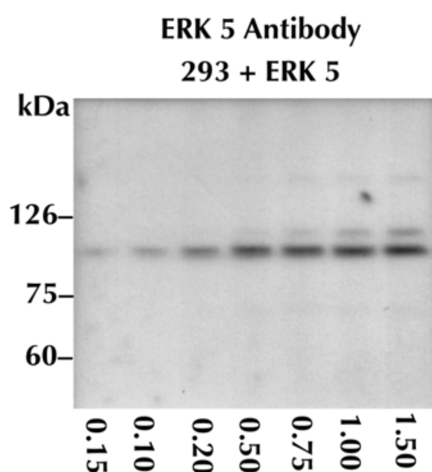
Karihaloo, A. et al. (2001) Differential MAPK pathways utilized for HGF- and EGF-dependent renal epithelial morphogenesis. J. Biol. Chem. 276(12):9166-9173 (cites the use of this antibody in Western blot analysis).

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

Marinissen, M.J. et al. (1999) A network of mitogen-activated protein kinases links G protein-coupled receptors to the c-jun promoter: a role for c-Jun NH2-terminal kinase, p38s, and extracellular signal-regulated kinase 5. Mol. Cell. Biol. 19(6):4289-4301.

Kamakura, S. et al. (1999) Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. J. Biol. Chem. 274(37):26563-26571.

Kato, Y. et al. (1998) Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. Nature 395(6703):713-716.



Titration

Extracts of HEK 293 cells transfected with recombinant human ERK5/BMK1 were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. The membrane was blocked with a 4% BSA-TBST buffer overnight at 4°C, then incubated with 0.15-1.5 µg/mL of the ERK5/BMK1 antibody. After washing, the membrane was incubated with goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase (ALI4405) and signals were detected by chemiluminescence using the Tropix WesternStar™ detection.

The data show detection of the full length recombinant ERK5/BMK1 protein over a wide range of antibody concentrations.

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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 (Invitrogen International catalog number 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.
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 concentration of 0.1-1.0 µg/mL in Tris buffered saline supplemented with 1% 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 (catalog number ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (catalog number 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 catalog number 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
4 gm Ig-free BSA
0.1 mL Tween 20

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