Rabbit (polyclonal)  
**Anti-ERK5/BMK1** 
**Unconjugated**

PRODUCT ANALYSIS SHEET

<table>
<thead>
<tr>
<th><strong>Catalog Number:</strong></th>
<th>44-688</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lot Number:</strong></td>
<td>See product label</td>
</tr>
<tr>
<td><strong>Quantity/Concentration:</strong></td>
<td>See product label</td>
</tr>
<tr>
<td><strong>Form of Antibody:</strong></td>
<td>Rabbit polyclonal immunoglobulins in phosphate buffer, pH 7.4.</td>
</tr>
<tr>
<td><strong>Preservation:</strong></td>
<td>0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)</td>
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<tr>
<td><strong>Specificity:</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Species Cross-Reactivity:</strong></td>
<td>This antibody cross-reacts with mouse (Karihaloo et al., 2001).</td>
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<tr>
<td><strong>Immunogen:</strong></td>
<td>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.</td>
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<tr>
<td><strong>Purification:</strong></td>
<td>Purified from rabbit serum by sequential epitope-specific chromatography.</td>
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<tr>
<td><strong>Applications:</strong></td>
<td>The antibody has been used for Western blot applications.</td>
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<tr>
<td><strong>Suggested Working Dilutions:</strong></td>
<td>For Western blot applications, we recommend using the antibody at 0.1-1.0 μg/mL. The optimal antibody concentration should be determined for each specific application.</td>
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<tr>
<td><strong>Storage:</strong></td>
<td>Store at ~80°C. Upon initial thawing, apportion into working aliquots and store at ~80°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody. The antibody is stable for at least 6 months when stored appropriately.</td>
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<tr>
<td><strong>Expiration Date:</strong></td>
<td>Expires one year from date of receipt when stored as instructed.</td>
</tr>
</tbody>
</table>

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PI44688

(Rev 11/08) DCC-08-1089
References:


Extracts prepared from HEK 293 cells transfected with recombinant human ERK5/BMK1 were resolved on a 10% Tris-glycine gel and transferred to nitrocellulose. Membranes were blocked with 1% BSA, followed by incubation with 0.15-1.5 μg/mL of anti-ERK5/BMK1 antibody, which recognizes the target protein independent of the phosphorylation state. After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase (ALI4405) and the signal was detected by chemiluminescence using the Tropix WesternStar™ detection method and Kodak BioMax™ ultraclear film. 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 10⁷ 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 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.

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% BSA and 0.1% Tween 20 for 2 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.

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**Cell Lysis Buffer**

**Formulation:**

- 10 mM Tris, pH 7.4
- 100 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 1 mM NaF
- 20 mM Na₂P₂O₇
- 2 mM Na₃VO₄
- 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 BSA
- 0.1 mL Tween 20

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