Technical Data Sheet

PE Mouse anti-MEK1

Product Information

Material Number: 560099
Alternate Name: MAPK/ERK kinase 1, EC 2.7.12.2, kinase MEK1, MAPKK1, PRKMK1
Size: 50 Tests
Vol. per Test: 20 µl
Clone: 25/MEK1
Immunogen: Human MEK1 Recombinant Protein
Isotype: Mouse IgG2a
Reactivity: QC Testing: Human
Reported Reactivity: Mouse, Rat, Dog, Chicken

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

MEK1 (MapK/ERK Kinase 1) is a 45-kDa member of the MEK family of dual specificity kinases. MEK is activated by a variety of cellular serine/threonine kinases including c-Raf, A-Raf, c-mos, and MEK Kinase-1. Activated MEK phosphorylates MAP kinase (ERK) at threonine and tyrosine residues. This results in activation of ERK and its signaling pathway. MEK is highly specific for ERK and various MEKs preferentially phosphorylate individual ERK isoforms. MEK1 only activates ERK1 and ERK2. This specificity may result from variations in ERK regions that are known as the phosphorylation lip and kinase backbone. MEK's localization is cytoplasmic, but mitogenic stimulation induces a mass translocation to the nucleus. Mechanisms behind this nuclear translocation remain unknown. However, MEK contains an N-terminal nuclear export signal (NES) that mediates its rapid exodus from the nucleus and restores its unstimulated cellular distribution.

The 25/MEK1 monoclonal antibody recognizes MEK1, regardless of phosphorylation status.

The specificity of this antibody conjugate for flow cytometric analysis was validated by confirming that RNA-mediated interference (RNAi) of the specific protein reduced the staining of the cells (see figure). Furthermore, the capacity of the RNAi to down-regulate the expression of the relevant protein was confirmed by western blot analysis.

Analysis of MEK1 in HeLaS3 cells. HeLaS3 cells were either transfected with MEK1 RNAi (open histogram) or untreated (shaded histogram). The cells were fixed (BD Cytofix™ Fixation buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with PE Mouse anti-MEK1 (Cat. No. 560099). Down-regulation of MEK1 expression is evident in the RNAi-transfected cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
This mAb was characterized by flow cytometry (Flow) and western blot analysis (WB) using these model systems:

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Cells</th>
<th>Treatment</th>
<th>Fixation</th>
<th>Perm buffer</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>Human</td>
<td>HeLaS3</td>
<td>RNAi</td>
<td>Cytofix</td>
<td>Perm III</td>
<td>Down-regulation</td>
</tr>
<tr>
<td>Flow</td>
<td>Human</td>
<td>PBMC</td>
<td></td>
<td>Cytofix</td>
<td>Perm I, II or III</td>
<td>Positive Staining</td>
</tr>
<tr>
<td>WB</td>
<td>Human</td>
<td>A431 Cell Lysate</td>
<td></td>
<td></td>
<td></td>
<td>45 kDa</td>
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</tbody>
</table>

**Application Notes**

**Application**

Intracellular staining (flow cytometry) Routinely Tested

**Recommended Assay Procedure:**

Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation.

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
<td>100 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>557870</td>
<td>Fix Buffer I</td>
<td>250 mL</td>
<td>(none)</td>
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<tr>
<td>558595</td>
<td>PE Mouse IgG2a, κ Isotype Control</td>
<td>50 Tests</td>
<td>MOPC-173</td>
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<tr>
<td>558052</td>
<td>Perm Buffer II</td>
<td>125 mL</td>
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<tr>
<td>557885</td>
<td>Perm/Wash Buffer I</td>
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<td>(none)</td>
</tr>
<tr>
<td>558050</td>
<td>Perm Buffer III</td>
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<td>(none)</td>
</tr>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
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</table>

**Product Notices**

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

**References**


