Technical Data Sheet

Purified Mouse Anti-MEK1

Product Information

Material Number: 610122
Size: 150 µg
Concentration: 250 µg/ml
Clone: 25/MEK1
Immunogen: Human MEK1 Recombinant Protein
Isotype: Mouse IgG2a
Reactivity: QC Testing: Human
Target MW: Tested in Development: Chicken, Dog, Frog, Mouse, Rat
45 kDa
Storage Buffer: Aqueous buffered solution containing BSA, glycerol, 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.

Western blot analysis of MEK1 on a A431 lysate (Cat. No 611447). Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the anti-MEK1 antibody.

Immunofluorescent staining of HeLa cells (ATCC CCL-2). Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-MEK1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U2OS (ATCC HTB-96) cells and can be used with either perm protocol (see Recommended Assay Procedure).

Preparation and Storage

Store undiluted at -20°C.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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For country-specific contact information, visit bdbiosciences.com/how_to_order/

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Application Notes

**Application**

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<td>Bioimaging</td>
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<tr>
<td>Immunohistochemistry</td>
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**Recommended Assay Procedure:**

**Bioimaging**

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 μl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 μl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   OR
   b. Add 100 μl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 μl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 μl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 μl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 μl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 μl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 μl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 μl per well of 2 μg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

**Bioimaging:** For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/certified_reagents.jsp

**Western blot:** For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

**Suggested Companion Products**

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>611447</td>
<td>A431 Cell Lysate</td>
<td>500 μg</td>
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<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Ig</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
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<tr>
<td>353219</td>
<td>BD Falcon™ 96-well Imaging Plate</td>
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</tr>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
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<tr>
<td>558050</td>
<td>Perm Buffer III</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

**References**


