Technical Data Sheet

# PE Mouse anti-MEK1

## Product Information

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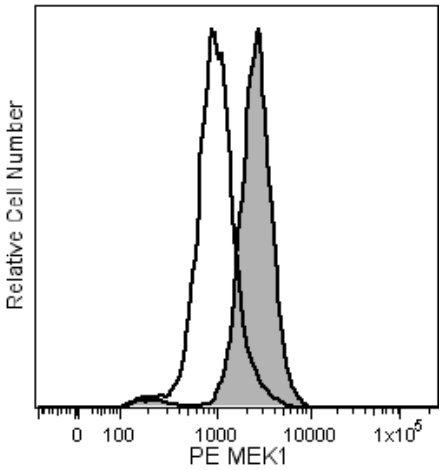
|---|---|
| **Material Number:** | **560099** |
| **Alternate Name:** | MAPK/ERK kinase 1, EC 2.7.12.2, kinase MEK1, MAPKK1, PRKMK1 |
| **Size:** | 50 tests |
| **Vol. per Test:** | 20 µl |
| **Clone:** | 25/MEK1 |
| **Immunogen:** | Human MEK1 Recombinant Protein |
| **Isotype:** | Mouse IgG2a |
| **Reactivity:** | Confirmed: Human<br>Reported: Chicken, Dog, Mouse, Rat |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

## Description

MEK1 (**M**apK/**E**RK **K**inase **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. 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

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

**BD Biosciences**

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## Application Notes

**Application**

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|---|---|
| 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.

This mAb was characterized by flow cytometry (Flow) and western blot analysis (WB) using these model systems:

| *Method* | *Species* | *Cells* | *Treatment* | *Fixation* | *Perm buffer* | *Result* |
|---|---|---|---|---|---|---|
| *Flow* | Human | HeLaS3 | RNAi | BD Cytofix™ | III | Down-regulation |
| *Flow* | Human | PBMC | Not Applicable | BD Cytofix™ | I, II or III | Positive Staining |
| *WB* | Human | A431 Cell Lysate | Not Applicable | Not Applicable | Not Applicable | 45 kDa |

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554655 | Fixation Buffer | 100 ml | (none) |
| 557870 | Fix Buffer I | 250 ml | (none) |
| 558595 | PE Mouse IgG2a, κ Isotype Control | 50 tests | MOPC-173 |
| 558050 | Perm Buffer III | 125 ml | (none) |
| 558052 | Perm Buffer II | 125 ml | (none) |
| 557885 | Perm/Wash Buffer I | 125 ml | (none) |

## Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. 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).
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.

## References


Aplin AE, Stewart SA, Assoian RK, Juliano RL. Integrin-mediated adhesion regulates ERK nuclear translocation and phosphorylation of Elk-1. *J Cell Biol.* 2001; 153(2):273-282. (Biology: Immunofluorescence, Western blot)

Gu J, Fujibayashi A, Yamada KM, Sekiguchi K. Laminin-10/11 and fibronectin differentially prevent apoptosis induced by serum removal via phosphatidylinositol 3-kinase/Akt- and MEK1/ERK-dependent pathways. *J Biol Chem.* 2002; 277(22):19922-19928. (Clone-specific: Western blot)

Robinson MJ, Cheng M, Khokhlatchev A, et al. Contributions of the mitogen-activated protein (MAP) kinase backbone and phosphorylation loop to MEK specificity. *J Biol Chem.* 1996; 271(47):29734-29739. (Biology)

Short SM, Boyer JL, Juliano RL. Integrins regulate the linkage between upstream and downstream events in G protein-coupled receptor signaling to mitogen-activated protein kinase. *J Biol Chem.* 2000; 275(17):12970-12977. (Clone-specific: Immunoprecipitation, In vitro kinase assay, Western blot)