

Technical Data Sheet

Alexa Fluor® 647 Mouse anti-MEK1

Product Information

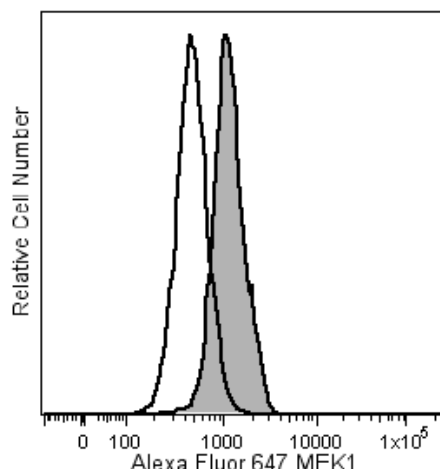
Material Number:	560101
Alternate Name:	MAPK/ERK kinase 1, EC 2.7.12.2, kinase MEK1, MAPKK1, PRKMK1
Size:	50 tests
Vol. per Test:	20 µl
Clone:	25/MEK1
Immunogen:	Human MEK1 Recombinant Protein
Isotype:	Mouse IgG2a
Reactivity:	Confirmed: Human Reported: Chicken, Dog, Mouse, Rat
Storage Buffer:	Aqueous 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 Alexa Fluor® 647 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Either BD Cytotfix™ 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:

<i>Method</i>	<i>Species</i>	<i>Cells</i>	<i>Treatment</i>	<i>Fixation</i>	<i>Perm buffer</i>	<i>Result</i>
<i>Flow</i>	Human	HeLaS3	RNAi	BD Cytotfix™	III	Down-regulation
<i>Flow</i>	Human	PBMC	Not Applicable	BD Cytotfix™	I, II or III	Positive Staining
<i>WB</i>	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)
558053	Alexa Fluor® 647 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/pharming/en/protocols for technical protocols.
2. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. 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).
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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. (Clone-specific: 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)