Technical Data Sheet

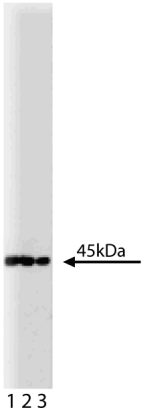
Purified Mouse Anti-MEK1

Product Information

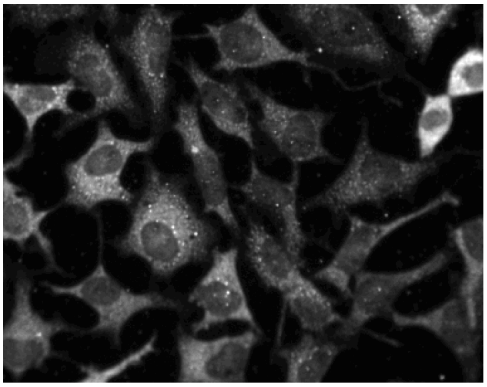
Material Number:	**610121**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	25/MEK1
Immunogen:	Human MEK1 Recombinant Protein
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Frog, Mouse, Rat
Target MW:	45 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, 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.



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 imager 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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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development

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/ceritifed_reagents.jsp

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
611447	A431 Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Triton is a trademark of the Dow Chemical Company.

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)

Freeman WM, Brebner K, Lynch WJ, et al. Changes in rat frontal cortex gene expression following chronic cocaine. *Brain Res Mol Brain Res.* 2002; 104(1):11-20. (Clone-specific: 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)