Technical Data Sheet

Purified Mouse Anti-MEK1

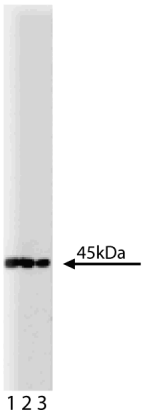
Product Information

Material Number:	**610122**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	25/MEK1
Immunogen:	Human MEK1 aa. 2-124
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Frog, Mouse, Rat
Target Molecular Weight:	45 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

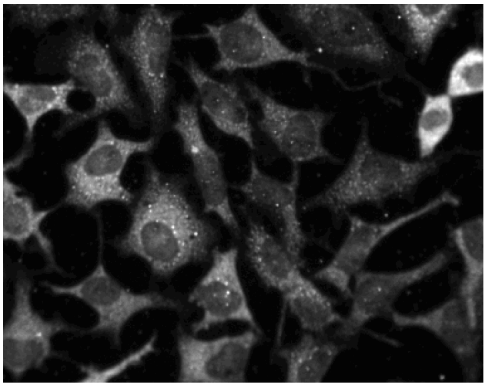
Description

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.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Western blot analysis of MEK1 on a A431 lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the anti-MEK1 antibody.*



Immunofluorescent staining of HeLa cells. *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 methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-MEK1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a Pathway 855 imager using a 20x objective. This antibody also stained A549 and U2OS cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

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Preparation and Storage

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

Application Notes

Application

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

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611447	A431 Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

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.

References

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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)

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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)