Technical Data Sheet

Alexa Fluor® 488 Mouse anti-MEK1 (pS298)

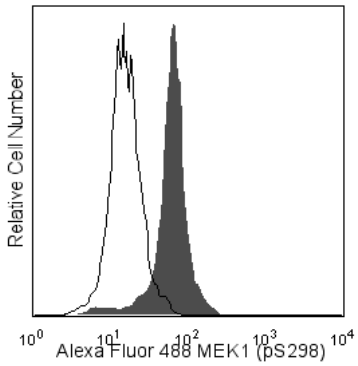
Product Information

Material Number:	**560192**
Alternate Name:	MAP2K1, MAPKK1, MKK1, MP2K1, PPKMK1
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J114-64
Immunogen:	Phosphorylated Human MEK1 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed by flow cytometry: Human Confirmed by western blot using purified antibody (Cat. No. 558375): Human, Mouse Predicted: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

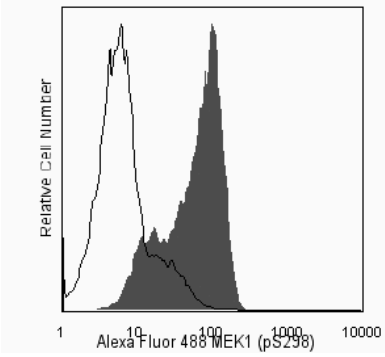
Description

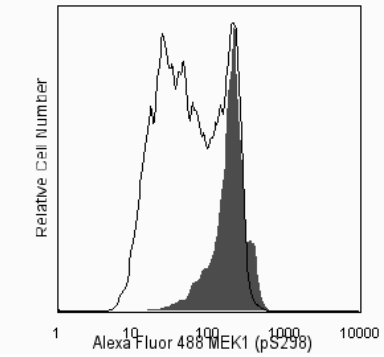
MEK (**M**ap/**E**rk **K**inase) 1 and 2 are serine/threonine kinases, also known as MAP kinase kinases (MAP2K1 and 2, MAPKK1 and 2, or MKK1 and 2). They activate the MAP (**M**itogen-**A**ctivated **P**rotein) kinases, also known as ERKs (**E**xtracellular signal **R**egulated **K**inases), which are critical kinases in multiple signal transduction pathways that regulate cell growth and differentiation. Activation of MEK 1 and 2 is dependent upon phosphorylation of serines 218 and/or 222 by activated MAP kinase kinase kinases (MAP3Ks), such as the Raf isoforms. Hormones, growth and differentiating factors, or tumor promoters induce Raf activation via activation of Ras proteins. Alternatively, cellular adhesion can lead to phosphorylation of MEK1 at serine 298 (S298), mediated by p21-activated kinase (PAK). The S298-phosphorylated MEK1 has an enhanced capacity to interact with Raf, resulting in MEK1 activation.

The J114-64 monoclonal antibody recognizes the phosphorylated S298 of MEK1.



Analysis of MEK1 (pS298) in human epithelioid carcinoma. *Serum-starved HeLa S3 cells (ATCC CCL 2.2) were either stimulated (shaded histogram) with 50 nM Calyculin A (Calbiochem, Cat. No. 208851) at 37˚C for 30 minutes or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37˚C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-MEK1 (pS298). Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*





Analysis of MEK1 (pS298) in human human peripheral blood lymphocytes (left panel) and monocytes (right panel). *Human peripheral blood mononuclear cells (PBMC) were either stimulated with 50 nM PMA (Sigma, P8139) for 15 minutes at 37˚C (shaded histogram) or unstimulated (open histogram). The PBMC were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-MEK1 (pS298). Lymphocytes and monocytes were selected by scatter profile. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

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

The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	HeLa S3	Calyculin A	Cytofix	Perm III	Up-regulation
	Human	PBMC	Calyculin A	Cytofix	Perm III	Up-regulation on lymphocytes and monocytes
	Human	PBMC	PMA	Cytofix	Perm III	Up-regulation on lymphocytes and monocytes
WB	Human	A-431	Lambda phosphatase			Loss of 45-kDa band
	Mouse	NIH/3T3	trypsin			45-kDa band down-regulated

Application Notes

Application

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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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).
3. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
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

Eblen ST, Slack JK, Weber MJ, Catling AD. Rac-PAK signaling stimulates extracellular signal-regulated kinase (ERK) activation by regulating formation of MEK1-ERK complexes. *Mol Cell Biol.* 2002; 22(17):6023-6033. (Biology)

Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J.* 2000; 351:289-305. (Biology)

Slack-Davis JK, Eblen ST, Zecevic M, et al. PAK1 phosphorylation of MEK1 regulates fibronectin-stimulated MAPK activation. *J Cell Biol.* 2003; 162(2):281-291. (Biology)