Technical Data Sheet

PE Mouse anti-MEK1 (pS298)

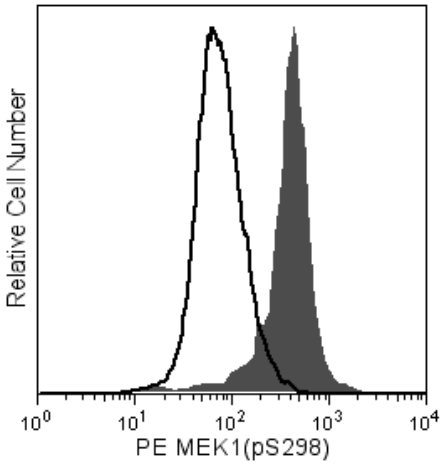
Product Information

Material Number:	**560042**
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 intracellular flow cytometry: Human
	Confrimed by western blot using purified antibody (Cat. No. 558375): Mouse
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 (**Mi**togen-**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. *Hela S3 cells (ATCC CCL 2.2) were serum starved overnight, detached using 1X trypsin, washed, resuspended in serum-free DMEM, and then either left unstimulated (open histogram) or stimulated (shaded histogram) with 50 nM Calyculin A (Calbiochem, Cat. No. 208851) at 37˚C for 30 minutes. 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 PE Mouse anti-MEK1 (pS298). Flow cytometry was performed on a BD™ FACSCalibur 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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Either BD Cytofix™ buffer or BD™ Phosflow Fix Buffer I may be used for cell fixation.

BD Biosciences

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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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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

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)