Technical Data Sheet

Alexa Fluor® 647 Mouse anti-MEK2

Product Information

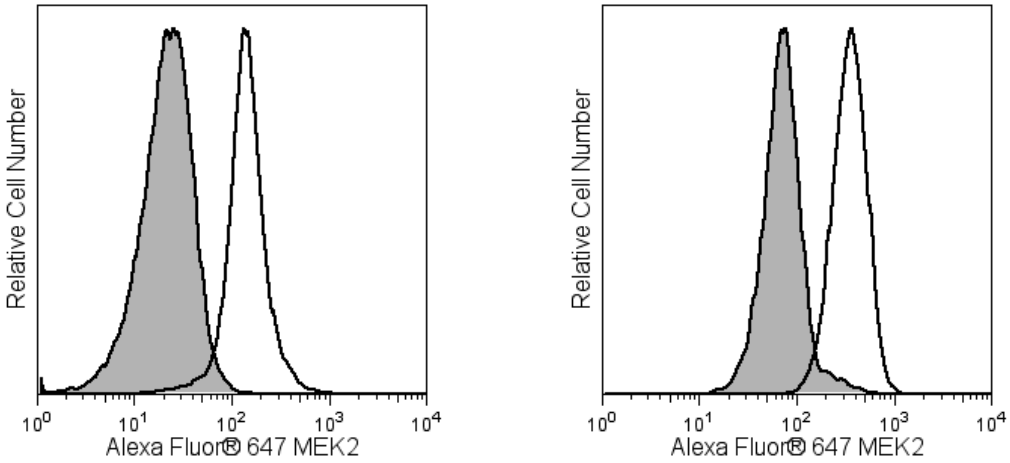
Material Number:	**560387**
Alternate Name:	MAP Kinase Kinase 2; ERK Kinase
Size:	50 tests
Vol. per Test:	20 µl
Clone:	96/MEK2
Immunogen:	Rat MEK2 aa. 1-110 Recombinant Protein
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in development by western blot using purified antibody: chicken, dog, frog, mouse, 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. MEK2 is seven amino acids larger and shares 81% identity with MEK1. In cultured cells, MEK2 is activated by serum. In vitro, v-Raf phosphorylates and activates MEK2. It is thought that all of these activated protein kinases are downstream of the Ras signal transduction pathway and represent an integral part of the Ras mitogenic signal.

The 96/MEK2 monoclonal antibody recognizes MEK2, 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).



LEFT: Analysis of MEK2 in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were lysed and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) for 10 minutes at 37ºC, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with either Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (Cat. No. 558053, shaded histogram) or Alexa Fluor® 647 Mouse anti-MEK2 (open histogram). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSArray™ flow cytometry system.*

RIGHT: Analysis of MEK2 in human epithelioid carcinoma. *HeLa S3 cells (ATCC CCL 2.2) were either transfected with MEK2 RNAi (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-MEK2. Flow cytometry was performed on a BD FACSArray™ 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® 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

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human cell lines and peripheral blood mononuclear cells using BD Cytofix™ Fixation Buffer. Any of the three BD™ Phosflow permeabilization buffers may be used; Perm Buffers II and III are preferred. We do not recommend using it for staining of whole blood using BD™ Phosflow Lyse/Fix Buffer.

Suggested Companion Products

Catalog Number	Name	Size	Clone
610235	Purified Mouse Anti-MEK2	50 µg	96/MEK2
610236	Purified Mouse Anti-MEK2	150 µg	96/MEK2
558053	Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control	50 tests	MOPC-173
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. 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).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Downey GP, Butler JR, Tapper H, et al. Importance of MEK in neutrophil microbicidal responsiveness. *J Immunol.* 1998; 160(1):434-443. (Biology)

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Tworkowski KA, Salghetti SE, Tansey WP. Stable and unstable pools of Myc protein exist in human cells. *Oncogene.* 2002; 21(55):8515-8520. (Biology)

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