Technical Data Sheet

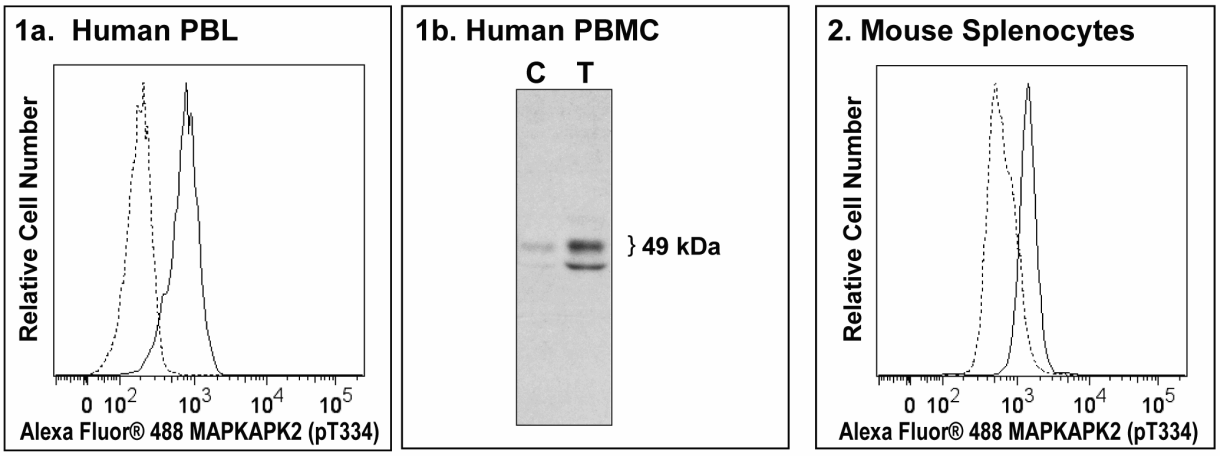
Alexa Fluor® 488 Mouse anti-MAPKAPK-2 (pT334)

Product Information

Material Number:	**562471**
Alternate Name:	MAP kinase-activated protein kinase 2, MAPKAP kinase 2; MK2; MAPK2
Size:	50 tests
Vol. per Test:	5 µl
Clone:	P24-694
Immunogen:	Phosphorylated Human MAPKAPK-2 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human; Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The P24-694 monoclonal antibody specifically binds to the phosphorylated T334 site (pT334) of MAPKAPK-2. MAPKAPK-2 is a serine/threonine protein kinase. This ~49 kDa member of the MAPKAPK family of protein kinases is also known as mitogen-activated protein kinase-activated protein kinase 2. MAPKAPK-2 is phosphorylated and activated by p38 MAP kinase in response to stress, cytokines and chemokines. MAPKAPK-2 is phosphorylated on multiple sites including Thr222, Ser272 and Thr334. Phosphorylation of any two of these three amino acid residues seems to be required for the activation of this kinase that serves multiple cellular functions. Phosphorylation of Thr334 was reported to be essential for nuclear export of the heterodimer formed between p38 MAPK and MAPKAPK-2. Mice deficient in MAPKAPK-2 have been shown to be protected from ischemic injury. MAPKAPK-2 is also reported to serve as a cell cycle checkpoint kinase in response to UV irradiation. The heat shock protein, HSP27 was shown to be one of the major substrates of MAPK and MAPKAPK-2.



Analyses of MAPKAPK2 (pT334) expression by Human and Mouse Cells.

Human Cells

Panel 1a: *Flow Cytometric analysis of MAPKAPK2 (pT334) in peripheral blood lymphocytes (PBL). Whole blood cells were not stimulated (dashed line histogram) or stimulated (solid line histogram) with 400 nM Phorbol 12-Myristate 13-Acetate (PMA; Sigma, Cat. No. P8139; 15 min, 37°C). Cells were fixed in 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049; 10 min, 37˚C) and permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice (30 min). Cells were stained with BD Phosflow™ Alexa Fluor® 488 Mouse Anti-MAPKAPK2 (pT334) (Cat. No. 562471) antibody. Fluorescence histograms showing MAPKAPK2 (pT334) expression were generated for gated events with the light scatter characteristics of intact lymphocytes using a BD FACSCanto™ II Flow Cytometer.*

Panel 1b: *Western blot analysis of MAPKAPK2 (pT334) expressed by peripheral blood mononuclear cells (PBMC). Lysates from 1X10^6 untreated (C) and PMA-treated (T) PBMC were blotted using Purified Mouse Anti-MAPKAPK2 (pT334) mAb (2 µg/ml, Cat. No. 562469), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system. MAPKAPK2 (pT334) were identified as ~49 kDa bands by Western blotting.*

Mouse Cells

Panel 2: *Splenocytes were not stimulated (dashed line histogram) or stimulated (solid line histogram) with PMA (50 nM, 15 min, 37°C). Cells were fixed, permeabilized, stained with Alexa Fluor® 488 Mouse anti-MAPKAPK2 (pT334) Ab and analyzed by flow cytometry as described above.*

Preparation and Storage

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

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

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

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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	Whole blood	PMA	Lyse/Fix	Perm I, II, III, or IV	Induced. All perm conditions allowed detection, with Perm/Wash Buffer I providing the best S/N.
	Human	PBMCs	PMA	Cytofix	Perm III	Induced
	Human	Jurkat	Anisomycin	Cytofix	Perm III	Induced
	Human	THP-1	Anisomycin + peptide blocking	Cytofix	Perm III	Induced. Blocked by pT334 phospho peptide but not by non-phospho peptide.
	Mouse	Spleen cells	PMA	Lyse/Fix	Perm III	Induced
WB	Human	PBMC	PMA			49-kDa band increased
	Human	Jurkat	Anisomycin			49-kDa band induced
	Human	THP-1	Anisomycin + peptide blocking			49-kDa band induced. Blocked by pT334 phospho peptide but not by non-phospho peptide.
	Mouse	Spleen cells	PMA			49-kDa band increased

Suggested Companion Products

Catalog Number	Name	Size	Clone
557782	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	50 tests	MOPC-21
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
562469	Purified Mouse anti-MAPKAPK-2 (pT334)	0.1 mg	P24-694
562470	PE Mouse anti-MAPKAPK-2 (pT334)	50 tests	P24-694
562472	Alexa Fluor® 647 Mouse anti-MAPKAPK-2 (pT334)	50 tests	P24-694

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. An isotype control should be used at the same concentration as the antibody of interest.
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 Multicolor Flow Cytometry 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Wang X, Xu L, Wang H, Young PR, Gaestel M, Feuerstein GZ.. Mitogen-activatedprotein kinase-activated protein (MAPKAP) kinase 2 deficiency protects brain fromischemic injury in mice. *J Biol Chem.* 2002; 277(46):43968. (Biology)

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