Technical Data Sheet

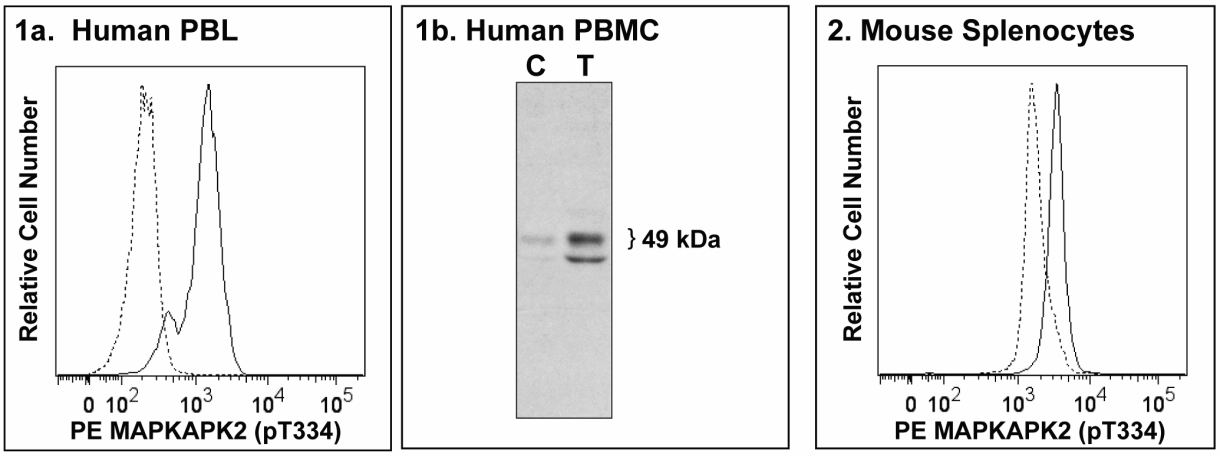
PE Mouse anti-MAPKAPK-2 (pT334)

Product Information

Material Number:	**562470**
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) expressed by 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 then stained with BD Phosflow™ PE Mouse Anti-MAPKAPK2 (pT334) (Cat. No. 562470) 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 System.*

Panel 1b: *Western blot analysis of MAPKAPK2 (pT334) expressed by human peripheral blood mononuclear cells (PBMC). Lysates from 1X10^6 untreated (C) and PMA-treated (T) PBMC were blotted using Purified Mouse Anti-MAPKAPK2 (pT334) antibody (2.0 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 PE Mouse anti-MAPKAPK2 (pT334) antibody and analyzed by flow cytometry as above.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
562469	Purified Mouse anti-MAPKAPK-2 (pT334)	0.1 mg	P24-694
562471	Alexa Fluor® 488 Mouse anti-MAPKAPK-2 (pT334)	50 tests	P24-694
562472	Alexa Fluor® 647 Mouse anti-MAPKAPK-2 (pT334)	50 tests	P24-694
554656	Stain Buffer (FBS)	500 ml	(none)
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21

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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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