Technical Data Sheet

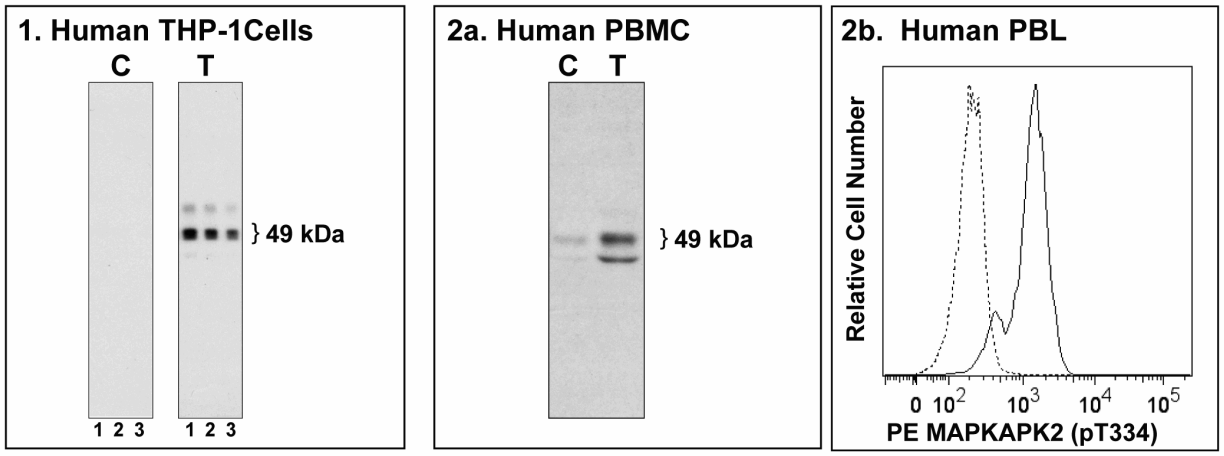
Purified Mouse anti-MAPKAPK-2 (pT334)

Product Information

Material Number:	**562469**
Alternate Name:	MAP kinase-activated protein kinase 2, MAPKAP kinase 2; MK2; MAPK2
Entrez Gene ID:	9261, 17164
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	P24-694
Immunogen:	Phosphorylated Human MAPKAPK-2 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Target MW:	~49 kDa
Storage Buffer:	Aqueous buffered solution containing ≤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.

Panel 1: *Western blot analysis of MAPKAPK2 (pT334) expressed by human THP-1 cells. Lysates (15 µg total cell protein/lane) from untreated (C) and PMA-treated (T) (Phorbol 12-Myristate 13-Acetate, Sigma, Cat. No. P8139; 50 nM, 15 min) THP-1 cells were blotted using Purified Mouse Anti-MAPKAPK2 (pT334) antibody (Cat. No. 562469; 0.5, 0.25, and 0.125 µg/ml for Lanes 1, 2, and 3, respectively), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system.*

Panel 2a: *Western blot analysis of MAPKAPK2 (pT334) expressed by human peripheral blood mononuclear cells (PBMC). Lysates from 1 million untreated (C) and PMA-treated (T) PBMC were blotted using Purified Mouse Anti-MAPKAPK2 (pT334) antibody (2.0 µg/ml) as described above.*

Panel 2b: *Flow Cytometric analysis of MAPKAPK2 (pT334) expressed by peripheral blood lymphocytes (PBL). Human whole blood cells were either not stimulated (dashed line histogram) or stimulated (solid line histogram) with 400 nM PMA (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™ PE Mouse Anti-MAPKAPK2 (pT334) (Cat. No. 562470) antibody. Fluorescence histograms showing MAPKAPK2 (pT334) expression were generated for gated events with the forward and side-light scatter characteristics of intact lymphocytes using a BD FACSCanto™ II Flow Cytometer System.*

Note: *MAPKAPK2 (pT334) were identified as ~49 kDa bands in the cell lysates by Western blotting.*

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Preparation and Storage

Store undiluted at 4°C.

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

Application Notes

Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development

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
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
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)
562470	PE Mouse anti-MAPKAPK-2 (pT334)	50 tests	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

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.

References

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Heidenreich O, Neininger A, Schratt G, et al. MAPKAP kinase 2 phosphorylates serum response factor in vitro and in vivo. *J Biol Chem.* 1999; 274(20):14434-14443. (Biology)

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