Technical Data Sheet

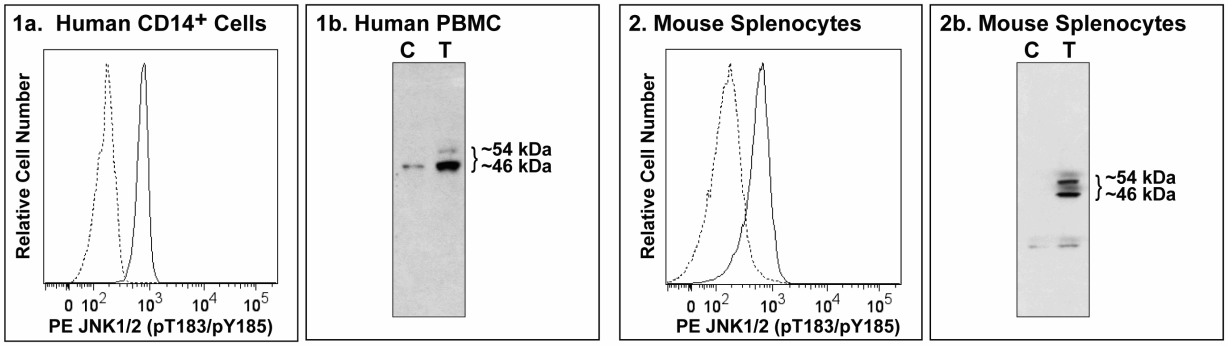
PE Mouse anti-JNK (pT183/pY185)

Product Information

Material Number:	**562480**
Alternate Name:	MAPK8, MAPK9; SAPK1, SAPK; PRKM8, PRKM9
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N9-66
Immunogen:	Phosphorylated Human JNK 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 N9-66 monoclonal antibody specifically binds to JNK1 and JNK2 phosphorylated at the pT183/pY185 sites. c-Jun NH2-terminal Kinases (JNKs), also called Stress Activated Protein Kinases (SAPKs), are mitogen-activated protein kinases (MAPKs) with observed molecular weights of ~46 kDa (JNK1) and ~54 kDa (JNK2). Along with the p38 and ERK families, JNK represents one of three major classes of MAPKs. Complete activation of JNK requires the phosphorylation of both Thr183 and Tyr185 that are located in a Thr-X-Tyr motif. Phosphorylation of these residues is carried out by MKK4 and MKK7 that are phosphorylated and activated by MEKKs and MLKs in response to stress signals delivered through small GTPases of the Rho family. Once activated, JNK can translocate into the nucleus and regulate the expression of genes through phosphorylation of c-Jun, ATF-2, and other transcription factors. JNK plays a role in signal transduction in response to cytokines and various forms of environmental stress, such as endotoxins, UV irradiation, heat, and hyperosmolarity. JNK is critical to the regulation of cell growth, apoptosis, and the cellular response to stress, making it an important factor in tumorigenesis and adaptive immunity. During antibody development, the N9-66 monoclonal antibody was found to detect phosphorylated JNK1/2 by Western blot analysis of cellular lysates and by immunofluorescent staining and flow cytometric analysis of fixed and permeabilized cells. This antibody crossreacts with phosphorylated JNK1/2 expressed by mouse cells, as tested by Western blot analysis and flow cytometry.



Analyses of JNK1/2 (pT183/pY185) expression by Human and Mouse Cells.

Human Cells

Panel 1a: *Flow cytometric analysis of JNK1/2 (pT183/pY185) expressed by human peripheral blood CD14+ cells. Whole blood cells were prestained with BD Horizon™ V450 Mouse Anti-Human CD14 (Cat. No. 560350/560349) and were then either not stimulated (dashed line histogram) or stimulated (solid line histogram) with PMA (Sigma, Cat. No. P8139; 400 nM), Ionomycin (Sigma, Cat. No. I0634, 250 ng/ml) and LPS (Sigma, Cat. No. L3137, 10 µg/m) at 37˚C for 15 minutes (ie, PMA/Iono/LPS treated). Cells were fixed in 1× 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-JNK (pT183/pY185) (Cat. No. 562480). Histograms showing JNK1/2 (pT183/pY185) expression were generated for CD14-positive gated events with the forward and side-light scatter characteristics of intact cells using a BD FACSCanto™ II Flow Cytometer System.*

Panel 1b: *Western blot analysis of JNK1/2 (pT183/pY185) expressed by peripheral blood mononuclear cells (PBMC). Lysates from 1 million untreated (C) and PMA/Iono/LPS-treated (T) PBMC were blotted using Purified Mouse Anti-JNK1/2 (pT183/pY185) antibody (2.0 µg/ml), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system. JNK1/2 (pT183/pY185) were identified as ~46 kDa and ~ 54 kDa bands, respectively.*

Mouse Cells

Panel 2a: *Flow cytometric analysis of JNK1/2 (pT183/pY185) expressed by mouse splenocytes. Splenocytes were either not stimulated (dashed line histogram) or were PMA/Iono/LPS treated (solid line histogram). Cells were fixed, permeabilized, stained and analyzed as described above.*

Panel 2b: *Western blot analysis of JNK1/2 (pT183/pY185) expressed by mouse splenocytes. Lysates from 1 x 10^6 untreated (C) and PMA/Iono/LPS-treated (T) splenocytes were blotted as described above.*

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Preparation and Storage

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

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.

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/Ionomycin/LPS + antibody blocking	Lyse/Fix	Perm III	Induced in monocytes and lymphocytes. Blocked by three anti-JNK (pT183/pY185) antibodies but not Ig isotype control.
	Human	Whole blood	PMA/Ionomycin	Lyse/Fix	Perm III	Induced in lymphocytes
	Human	Whole blood	LPS	Lyse/Fix	Perm III	Induced in monocytes
	Human	Whole blood	Anisomycin	Lyse/Fix	Perm III	Induced in monocytes
	Human	Jurkat	Anisomycin	Cytofix	Perm III	Induced
	Mouse	Spleen cells	PMA/Ionomycin/LPS	Lyse/Fix	Perm III	Induced
WB	Human	PBMC	PMA/Ionomycin/LPS			46-kDa and 54-kDa bands induced
	Human	PBMC	PMA			46-kDa and 54-kDa bands induced
	Human	Jurkat	Anisomycin + peptide blocking			46-kDa and 54-kDa bands induced. Blocked by pT183/pY185 phospho peptide but not by non-phospho peptide.
	Human	Thp1	Anisomycin			46-kDa and 54-kDa bands induced
	Mouse	Spleen cells	PMA/Ionomycin/LPS			46-kDa and 54-kDa bands induced

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560350	V450 Mouse Anti-Human CD14	30 tests	MφP9
560349	V450 Mouse Anti-Human CD14	120 tests	MφP9
554656	Stain Buffer (FBS)	500 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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

Fleming Y, Armstrong CG, Morrice N, Paterson A, Goedert M, Cohen P. Synergistic activation of stress-activated protein kinase 1/c-Jun N-terminal kinase (SAPK1/JNK) isoforms by mitogen-activated protein kinase kinase 4 (MKK4) and MKK7. *Biochem J.* 2000; 352:145-154. (Biology)

Huang G, Shi LZ, Chi H. Regulation of JNK and p38 MAPK in the immune system: signal integration, propagation and termination. *Cytokine.* 2009; 48(3):161-169. (Biology)

Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev.* 2001; 81(2):807-869. (Biology)

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