Technical Data Sheet

Alexa Fluor® 647 Mouse Anti-p38 MAPK (pT180/pY182)

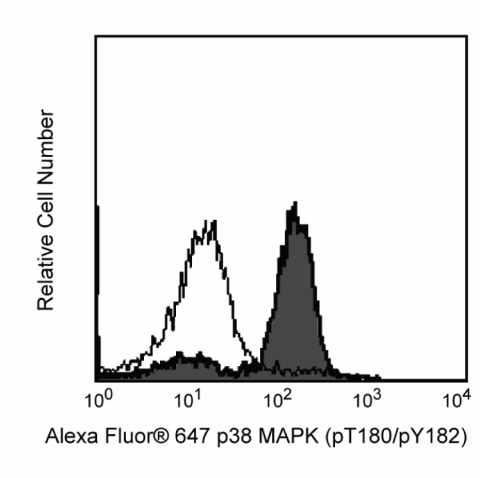
Product Information

Material Number:	**562066**
Alternate Name:	MK14, 11, 12, 13; CSBP1; SAPK2, 2A, 3, 4; MX12, ERK-6, ERK5
Size:	250 tests
Vol. per Test:	5 µl
Clone:	36/p38 (pT180/pY182)
Immunogen:	Phosphorylated Human p38 MAPK (pT180/pY182) Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development by western blotting using the purified antibody: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Activation of the immune and inflammatory responses often involves the recognition of bacterial endotoxin (lipopolysaccharide or LPS). Binding of LPS by monocytes results in the production and release of proinflammatory cytokines, such as IL-1 and TNF. LPS-induced signaling cascades involve members of the Ser/Thr protein kinase family known as the **M**itogen **A**ctivated **P**rotein **K**inases (MAPKs). MAPK signal transduction pathways mediate the effects of various extracellular stimuli on biological processes such as proliferation, differentiation, and death. The p38 MAPKs include p38α (MAPK14), β (MAPK11), γ (MAPK12), and δ (MAPK13). These Ser/Thr kinases are activated by dual phosphorylation on threonine (T) and tyrosine (Y) within the motif Thr-Gly-Tyr located in kinase subdomain VIII. Activation of p38 MAPK is mediated specifically by the *M*AP *K*inase *K*inases, MKK3, MKK4, and MKK6. This leads to the activation of multiple transcription factors (NF-κB, ATF-2, Elk-1, and CHOP) that induce expression of many different genes, including proinflammatory cytokine genes. Thus, p38 MAPKs are central kinases in multiple signal transduction pathways.

The 36/p38 (pT180/pY182) monoclonal antibody recognizes the conserved dual phosphorylated site pT180/pY182 of p38α, β, γ, and δ.



Flow cytometric analysis of p38 MAPK (pT180/pY182). *Human peripheral blood mononuclear cells (PBMCs) were either left unstimulated (unshaded) or stimulated (shaded) with 40 nM PMA for 10 minutes at 37°C. Cells were fixed with BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37°C and then permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) for 30 minutes on ice. Cells were then washed twice in BD Pharmingen™ Stain Buffer, and then stained with the Alexa Fluor® 647 mouse anti-p38 MAPK (pT180/pY182) antibody. The cells were analyzed on a BD FACSCalibur™ flow cytometer. For intracellular staining of human whole blood, BD Phosflow™ Lyse/Fix buffer (Cat. No. 558049) may be used for fixation.*

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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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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	PBMC	PMA	Cytofix	Perm I, II, or III	Weak induction observed
	Human	Whole Blood	PMA	Lyse/Fix	Perm III	Weak induction observed
	Human	PBMC	LPS or Anisomycin	Cytofix	Perm I, II, or III	Greater induction on monocytes than lymphocytes
WB	Human	HeLa	Anisomycin			38-42-kDa band induced
	Human	PBMC	Anisomycin			38-42-kDa band induced

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

For more information about BD Phosflow™: Please refer to http://www.bdbiosciences.com/support/resources/phosflow/index.jsp

Investigators may also find the following protocols to be helpful:

Phosflow protocol for human PBMC: Please refer to http://www.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_PBMCs.pdf
Phosflow protocol for human whole blood: Please refer to
http://www.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_Whole_Blood_Samples.pdf
Phosflow protocol for adherent cells: Please refer to http://www.bdbiosciences.com/support/resources/protocols/protocol_adherent.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
612288	Purified Mouse Anti-p38 MAPK (pT180/pY182)	50 µg	36/p38 (pT180/pY182)
612289	Purified Mouse Anti-p38 MAPK (pT180/pY182)	150 µg	36/p38 (pT180/pY182)
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. 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

Brunet A, Pouyssegur J. Identification of MAP kinase domains by redirecting stress signals into growth factor responses. *Science.* 1996; 272(5268):1652-1655. (Biology)

Han J, Lee JD, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science.* 1994; 265(5173):808-811. (Biology)

Winston BW, Chan ED, Johnson GL, Riches DW. Activation of p38mapk, MKK3, and MKK4 by TNF-alpha in mouse bone marrow-derived macrophages. *J Immunol.* 1997; 159(9):4491-4497. (Biology)