Technical Data Sheet

PE-Cy™7 Mouse anti-p38 MAPK (pT180/pY182)

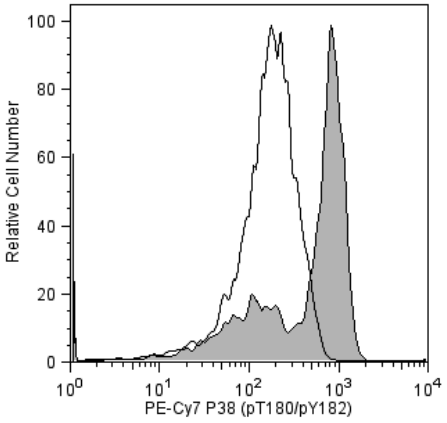
Product Information

Material Number:	**560241**
Alternate Name:	MAPK14; p38 MAP kinase; p38 MAPK; RK; CSBP2
Size:	50 Tests
Vol. per Test:	20 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: 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.



Analysis of p38 MAPK (pT180/pY182) in monocytes. *Human peripheral blood mononuclear cells (PBMC) were either stimulated with 40 µM Anisomycin for 20 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE-Cy™7 Mouse anti-p38 MAPK (pT180/pY182). Monocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCanto™ II flow cytometer.*

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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were 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:

Either BD Cytofix™ fixation buffer or BD™ Phosflow Fix Buffer I may be used for cell fixation. Any of the three BD™ Phosflow permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
557870	Fix Buffer I	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)
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)

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. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Cy is a trademark of Amersham Biosciences Limited.
5. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
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.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
10. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

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)

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