Technical Data Sheet

PE-CF594 Mouse Anti-p38 MAPK (pT180/pY182)

Product Information

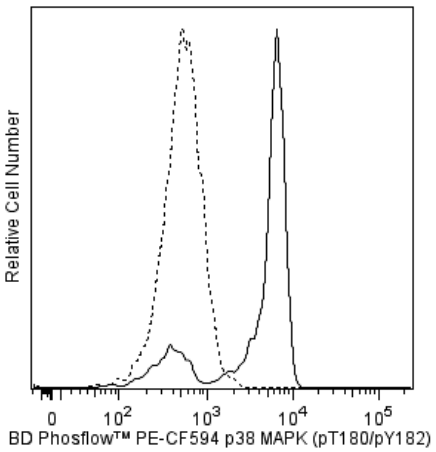
Material Number:	**563569**
Alternate Name:	MAPK14; p38 MAP kinase; p38 MAPK; RK; CSBP2
Size:	50 tests
Vol. per Test:	5 µl
Clone:	36/p38 (pT180/pY182)
Immunogen:	Phosphorylated Human p38 MAPK (pT180/pY182) Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
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.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Flow cytometric analysis of p38 MAPK (pT180/pY182) expression in monocytes. *Human peripheral blood mononuclear cells were either untreated (dashed line histogram) or treated (solid line histogram) by culture with 40 µM Anisomycin (Calbiochem, Cat. No. 176880) for 25 minutes at 37°C. The cells were fixed (10 min, 37°C) with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and then permeabilized (on ice, 30 min) with BD Phosflow™ Perm Buffer III, Cat. No. 558050). Cells were washed twice in BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) and then stained with BD Phosflow™ PE-CF594 Mouse Anti-p38 MAPK (pT180/pY182) antibody (Cat. No. 563569). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact monocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. 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.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
11. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
12. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
13. 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)

Perez OD, Mitchell D, Campos R, Gao GJ, Li L, Nolan GP. Multiparameter analysis of intracellular phosphoepitopes in immunophenotyped cell populations by flow cytometry. *Curr Protoc Cytom.* 2005; :6.20.1-6.20.22. (Clone-specific: Flow cytometry)

Suni MA, Maino VC. Flow cytometric analysis of cell signaling proteins. *Methods Mol Biol.* 2011; 717:155-169. (Clone-specific: Flow cytometry)

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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