Technical Data Sheet

Purified Mouse Anti-p38 MAPK (pT180/pY182)

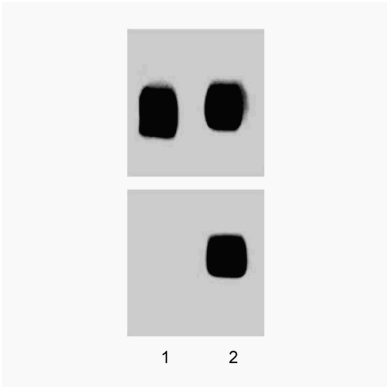
Product Information

Material Number:	**612289**
Alternate Name:	MK14, 11, 12, 13; CSBP1; SAPK2, 2A, 3, 4; MX12, ERK-6, ERK5
Size:	150 µg
Concentration:	250 µg/ml
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
Target MW:	38-42 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

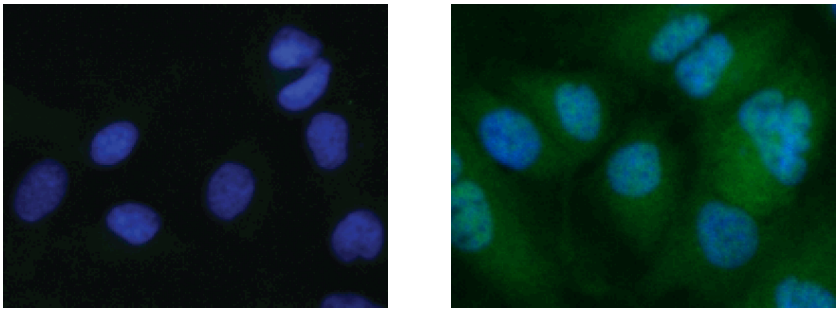
Description

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.



Western blot analysis for p38 MAPK (pT180/pY182). *HeLa cells (Human cervical epitheloid carcinoma; ATCC CCL-2) were either left untreated (lane 1) or treated (lane 2) with 25 µg/ml anisomycin, an antibiotic and protein synthesis inhibitor known to activate signal transduction pathways, for 15 minutes at 37°C. The top panel was probed with a mouse anti-p38α antibody (Cat. No. 611532) and the bottom was probed with the mouse anti-p38 MAPK (pT180/pY182) antibody at a 1:2500 dilution. The target band in each panel is observable at 38-42 kD.*



Immunofluorescent staining of HeLa cells. *HeLa cells (Human cervical epitheloid carcinoma; ATCC CCL-2) were seeded in a BD Falcon™ 96-well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were either left untreated (left image) or exposed to anisomycin for 30 minutes (right image). After treatment cells were stained using the alcohol perm protocol and the mouse anti-p38 MAPK (pT180/pY182) antibody. The second step reagent was Alexa-Fluor® 488 goat anti-mouse IgG (Invitrogen). Phospho p38 staining is pseudo-colored green, nuclei were stained with Hoechst 33342 and are pseudo-colored blue. Note the lack of staining for phospho-p38 in the untreated cells (left) and the nuclear/cytoplasmic staining in the stimulated cells (right). The images were captured on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) cells and can be used with either the Triton™ X-100 or alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

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

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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

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

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   OR b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate 1 hour.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark 1 hour.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
611692	HeLa + Anisomycin Cell Lysate	500 µg	(none)
612168	Purified Mouse Anti-p38α	50 µg	27/p38α/SAPK2a
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

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. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
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.
6. Triton is a trademark of the Dow Chemical Company.

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)