Store at 4°C

#12207

MAPK Family Alexa Fluor® 647 Conjugated Antibody Sampler Kit

☑ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 10/27/14

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Isotype	Flow Cytometry Dilution
Phospho-p38 MAPK (Thr180/Tyr182) (28B10) Mouse mAb (Alexa Fluor® 647 Conjugate)	4552	40 µl	Mouse IgG1	1:50
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (Alexa Fluor® 647 Conjugate)	13148	40 µl	Rabbit IgG	1:50
p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (Alexa Fluor® 647 Conjugate)	5376	40 µl	Rabbit IgG	1:50
Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb (Alexa Fluor® 647 Conjugate)	9257	40 µl	Mouse IgG1	1:50
Mouse (MOPC-21) mAb IgG1 Isotype Control (Alexa Fluor® 647 Conjugate)	4843	40 µl	Mouse IgG1	N/A
Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate)	2985	40 µl	Rabbit IgG	N/A

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The MAPK Family Alexa Fluor® 647 Conjugated Antibody Sampler Kit provides an economical means to study the activation status of members of the MAPK family of proteins without the need for a fluorescent secondary antibody.

Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs, such as cell proliferation, differentiation, motility, and death. p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase that participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). The p44/42 MAPK (Erk1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (5-7), and research investigators consider it an important target in the diagnosis and treatment of cancer (8). The stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) is potently and preferentially activated by a variety of environmental stresses including UV and gamma radiation, ceramides, inflammatory cytokines, and in some instances, growth factors and GPCR agonists (9).

Specificity/Sensitivity: Phospho-p38 MAPK (Thr180/Tyr182) (28B10) Mouse mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of p38 MAP kinase only when dually phosphorylated at Thr180 and Tyr182. This antibody does not appreciably cross-react with the corresponding phosphorylated forms of either p44/42 MAPK (Erk1/2) or SAPK/JNK. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of p44 and p42 MAPK (Erk1/2) when either dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), or singly phosphorylated at Thr202. This antibody does not cross-react with the corresponding phosphorylated residues of either SAPK/JNK or p38 MAP kinases. p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of total p44/42 MAPK (Erk1/2) protein. This antibody does not cross-react with SAPK/JNK or p38 MAP kinases. Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of p46 and p54 SAPK/JNK dually phosphorylated at Thr183 and Tyr185. This antibody does not recognize endogenous levels of phosphorylated p44/42 or p38 MAP kinases.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr180/Tyr182 of human p38 MAP kinase protein, a synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase protein, a synthetic peptide corresponding to residues near the carboxy terminus of rat p44 MAP kinase protein, or a synthetic phosphopeptide corresponding to residues around Thr183/Tyr185 of human SAPK/JNK protein.

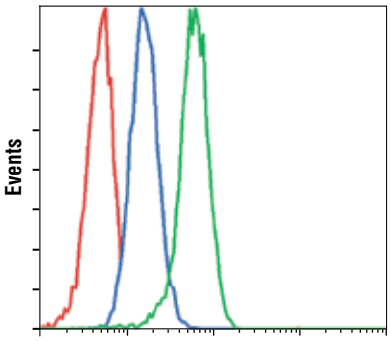
Background References:

(1) Rouse, J. et al. (1994) *Cell* 78, 1027-37.
(2) Han, J. et al. (1994) *Science* 265, 808-11.
(3) Lee, J.C. et al. (1994) *Nature* 372, 739-46.
(4) Freshney, N.W. et al. (1994) *Cell* 78, 1039-49.
(5) Roux, P.P. and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320-44.
(6) Baccarini, M. (2005) *FEBS Lett* 579, 3271-7.
(7) Meloche, S. and Pouysségur, J. (2007) *Oncogene* 26, 3227-39.
(8) Roberts, P.J. and Der, C.J. (2007) *Oncogene* 26, 3291-310.
(9) Davis, R.J. (1999) *Biochem Soc Symp* 64, 1-12.

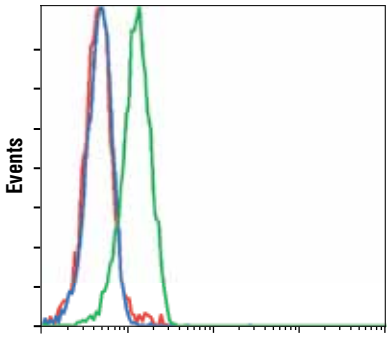
Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. *Do not aliquot the antibodies. Protect from light. Do not freeze.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

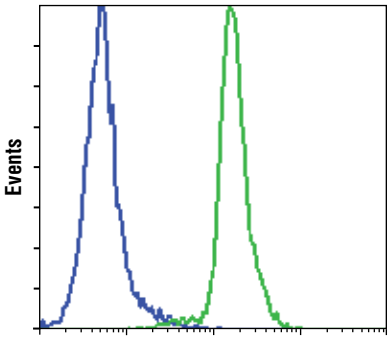
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



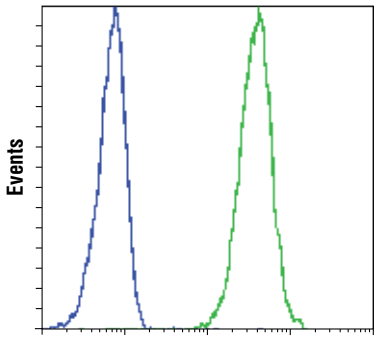
Flow cytometric analysis of THP-1 cells, untreated (blue) or anisomycin-treated (green), using ***Phospho-p38 MAPK (Thr180/Tyr182) (28B10) Mouse mAb (Alexa Fluor® 647 Conjugate) #4552*** *compared to a nonspecific control antibody (red).*



Flow cytometric analysis of THP-1 cells, untreated (blue) or anisomycin-treated (green), using ***Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb (Alexa Fluor® 647 Conjugate) #9257*** *compared to a nonspecific control antibody (red).*



Flow cytometric analysis of Jurkat cells using ***p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (Alexa Fluor® 647 Conjugate) #5376*** *(green) compared to Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) #2985 (blue).*



*Flow cytometric analysis of Jurkat cells treated with U0126 #9903 (blue) or TPA #4174 (green) using P****hospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (Alexa Fluor® 647 Conjugate) #13148****.*

#12207

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at –20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x10^6 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.
A-11070 Alexa Fluor® 488 F(ab')2 fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
A-11017 Alexa Fluor® 488 F(ab')2 fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)