Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb



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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype	
W, IP, IF-IC, F	H, M, R, Hm, Sc	46 kDa, 54 kDa	Mouse IgG1**	

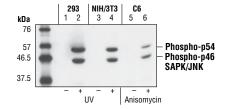
Background: The stress-activated protein kinase/Junamino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses including UV and gamma radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-MEKK4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4/7. Upon activation, MKKs phosphorylate and activate the SAPK/JNK kinase (2). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (3). Both Rac1 and cdc42 mediate the stimulation of MEKKs and MLKs (3). Alternatively, MKK4/7 can be activated in a GTPase independent mechanism via stimulation of a cerminal center kinase (GCK) family member (4). There are three SAPK/JNK genes each of which undergoes alternative splicing resulting in numerous isoforms (3). SAPK/JNK, when active as a dimer, can translocate to the nucleus and regulate transcription through its effects on c-Jun, ATF-2 and other transcription factors (3,5).

Specificity/Sensitivity: Phospho-SAPK/JNK (Thr183/ Tyr185) (G9) Mouse mAb detects 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 MAPK or p38 MAP kinase.

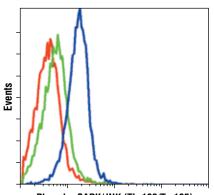
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr183/Tyr185 of human SAPK/JNK.

Background References:

- (1) Davis, R.J. (1999) Biochem Soc Symp 64, 1-12.
- (2) Ichijo, H. (1999) Oncogene 18, 6087-93.
- (3) Kyriakis, J.M. and Avruch, J. (2001) *Physiol Rev* 81, 807-69.
- (4) Kyriakis, J.M. (1999) J Biol Chem 274, 5259-62.
- (5) Leppä, S. and Bohmann, D. (1999) *Oncogene* 18, 6158-62.
- (6) Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Bio-chem Sci* 23, 481-5.



Western blot analysis of extracts from 293 cells, untreated or UV-treated (lanes 1 and 2), NIH/3T3 cells, untreated or UV-treated (lanes 3 and 4) and C6 cells, untreated or anisomycin-treated (lanes 5 and 6), using Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb.



Phospho-SAPK/JNK (Thr183/Tyr185)

Flow cytometric analysis of Jurkat cells, untreated (green) or anisomycin-treated (blue), using Phospho-SAPK/JNK (Thr183/ Tyr185) (G9) Mouse mAb compared to a nonspecific negative control antibody (red). Entrez-Gene ID #5599 Swiss-Prot Acc. #P45983

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibody.*

*Species cross-reactivity is determined by western blot.

**Anti-mouse secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

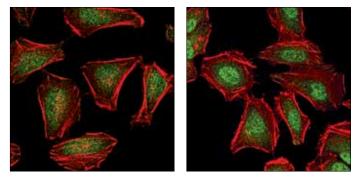
Western Blotting	1:2000
Immunoprecipitation	1:250
Flow Cytometry	1:400
Immunofluorescence (IF-IC)	1:200

For application specific protocols please see the web page for this product at www.cellsignal.com.

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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.



Confocal immunofluorescent analysis of HeLa cells untreated (left) and anisomycin-treated (right) using Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red).