Revision 1

#7103 Store at -20C

PathScan® Multi-Target HCA Stress and Apoptosis Kit

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Kit Includes*	Quantity	Applications	Dilution	Isotype
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-MAPKAPK-2 (Thr334) (27B7) Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-HSP27 (Ser82) Antibody II	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb	140 µl	HCA, ICW, IF-IC	1:10	Mouse IgG1
Phospho-c-Jun (Ser73) (D47G9) XP™ Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	140 µl	HCA, ICW, IF-IC	1:10	Mouse IgG1
Cleaved Caspase-3 (Asp175) Antibody	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Cleaved PARP (Asp214) Antibody (Human Specific)	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG

*Component formulation specific to kit.

Applications Key: HCA=High Content Analysis, ICW=In-Cell Western, IF-IC=Immunofluorescence (Immunocytochemistry)

Description

CST's PathScan® Multi-Target HCA Stress and Apoptosis Kit contains eight primary antibodies that target cellular stress and apoptotic signaling pathways. This kit is designed to elucidate the signaling occurring through key pathway nodes using automated imaging or laser scanning platforms or manual immunofluorescent microscopy. The kit provides the investigator with a quick and easy means to choose the endpoints that will be the most robust and useful for subsequent studies, whether large high content/high throughput screening projects or single small-scale experiments. The antibodies are supplied at 10X of their optimal dilution for immunofluorescent applications. This allows the antibodies to be easily diluted to their 1X working concentrations and dispensed into multi-well plates or slides. 140 µl of each antibody is supplied, which is sufficient for 24 wells on 96-well plates (50 µl 1X per well) or one row on two 96-well plates.

Storage

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

Background

Cellular stress and apoptosis involve a complex network of signaling pathways that maintain cellular homeostasis when confronted with a variety of potentially damaging effectors, including UV and gamma radiation, chemotherapeutic agents, osmotic shock, inflammatory cytokines, and other environmental stresses. The manner in which cells respond to stress has become an important metric in the study of disease due to the potential deregulation of these pathways in disease states. For example, cancer cells can affect these pathways to promote cell growth and metastasis (1). Some of the key members involved in stress-activated signaling belong to the mitogen-activated protein kinase (MAPK) pathway. The stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) is one such member that is potently and preferentially activated by a variety of environmental stresses (2-7). SAPK/JNK, when active as a dimer, can translocate to the nucleus where it regulates transcription through its effects on transcription factors such as c-Jun (4,6). Activation of c-Jun by phosphorylation at Ser63 and Ser73 through SAPK/JNK affects a diverse array of biological functions including cell proliferation, differentiation, and apoptosis (8). Similar to the SAPK/JNK pathway, p38 MAPK is activated by a variety of cellular stresses (9-13). When phosphorylated at Thr180 and Tyr182, p38 MAPK has been shown to activate MAP kinase-activated protein kinase 2 (MAPKAPK-2) and the transcription factors ATF-2, Max, and MEF2 (11-16). Phosphorylation at Thr222, Ser272, and Thr334 appears to be essential for the activity of MAPKAPK-2 (17), which can result in the phosphorylation of heat shock protein (HSP) 27 at Ser15, Ser78, and Ser82 (9,18). HSP27 is one of the small HSPs that are constitutively expressed at

different levels in various cell types and tissues. In response to stress, the expression level of HSP27 increases several-fold to confer cellular resistance to the adverse environmental change (18). The SAPK/JNK and p38 MAPK pathways also contribute to cell cycle checkpoint control through the activation of the p53 tumor suppressor protein, which plays a major role in cellular response to DNA damage and other genomic aberrations (19). Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (20). Stress-activated pathways also control the transcription of apoptotic proteins and mediators, thereby playing an important role in apoptosis and cell survival. Apoptosis is a regulated cellular suicide mechanism characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation (21). Cell survival requires the active suppression of apoptosis, which is accomplished by inhibiting the expression of pro-apoptotic factors as well as promoting the expression of anti-apoptotic factors. Caspases, a family of cysteine proteases, are the central regulators of apoptosis. Initiator caspases (including caspase-2, -8, -9, -10, -11, and -12) are closely coupled to pro-apoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including caspase-3, -6, and -7), which in turn execute apoptosis by cleaving cellular proteins following specific asparagine residues (1). Caspase-3 is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (22). PARP appears to be involved in DNA repair in response to environmental stress (23). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (24).

Background References

1. Herr, I. and Debatin, K.M. (2001) *Blood* 98, 2603-14.
2. Davis, R.J. (1999) *Biochem Soc Symp* 64, 1-12.
3. Ichijo, H. (1999) *Oncogene* 18, 6087-93.
4. Kyriakis, J.M. and Avruch, J. (2001) *Physiol Rev* 81, 807-69.
5. Kyriakis, J.M. (1999) *J Biol Chem* 274, 5259-62.
6. Leppä, S. and Bohmann, D. (1999) *Oncogene* 18, 6158-62.
7. Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Biochem Sci* 23, 481-5.
8. Davis, R.J. (2000) *Cell* 103, 239-52.
9. Rouse, J. et al. (1994) *Cell* 78, 1027-37.
10. Han, J. et al. (1994) *Science* 265, 808-11.
11. Lee, J.C. et al. *Nature* 372, 739-46.
12. Freshney, N.W. et al. (1994) *Cell* 78, 1039-49.
13. Raingeaud, J. et al. (1995) *J Biol Chem* 270, 7420-6.
14. Zervos, A.S. et al. (1995) *Proc Natl Acad Sci U S A* 92, 10531-4.
15. Zhao, M. et al. (1999) *Mol Cell Biol* 19, 21-30.
16. Yang, S.H. et al. (1999) *Mol Cell Biol* 19, 4028-38.
17. Ben-Levy, R. et al. (1995) *EMBO J* 14, 5920-30.
18. Landry, J. et al. (1992) *J Biol Chem* 267, 794-803.
19. Reinhardt, H.C. and Yaffe, M.B. (2009) *Curr Opin Cell Biol* 21, 245-55.
20. Levine, A.J. (1997) *Cell* 88, 323-31.
21. Elmore, S. (2007) *Toxicol Pathol* 35, 495-516.
22. Fernandes-Alnemri, T. et al. (1994) *J Biol Chem* 269, 30761-4.
23. Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-8.
24. Oliver, F.J. et al. (1998) *J Biol Chem* 273, 33533-9.

Trademarks and Patents

Limited Uses

respect to any third party products or services used by Customer in connection with the Products.