Store at -20°C

#8357

Stress and Apoptosis Antibody Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb	9664	20 µl	17, 19 kDa	Rabbit IgG
Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb	5625	20 µl	89 kDa	Rabbit IgG
Phospho-HSP27 (Ser82) (D1H2) XP® Rabbit mAb	9709	20 µl	27 kDa	Rabbit IgG
Phospho-c-Jun (Ser73) (D47G9) XP® Rabbit mAb	3270	20 µl	48 kDa	Rabbit IgG
Phospho-MAPKAPK-2 (Thr334) (27B7) Rabbit mAb	3007	20 µl	49 kDa	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	9286	20 µl	53 kDa	Mouse IgG1
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP® Rabbit mAb	4511	20 µl	43 kDa	Rabbit IgG
Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb	4668	20 µl	46, 54 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Description: The Stress and Apoptosis Antibody Sampler Kit provides an economical means of evaluating stress and apoptotic responses of each protein. The kit contains enough primary antibody to perform two western blot experiments per primary antibody.

Background: Cells respond to environmental or intracellular stresses through various mechanisms ranging from initiation of prosurvival strategies to activation of cell death pathways that remove damaged cells from the organism. Many of the proteins and cellular processes involved in normal signaling and survival pathways also play dual roles in cell death-promoting mechanisms. Apoptosis is a regulated cellular suicide mechanism characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation. Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) 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) (1). PARP appears to be involved in DNA repair in response to environmental stress (2). This protein can be cleaved by many ICE-like caspases *in vitro* (3,4) and is one of the main cleavage targets of caspase-3 *in vivo* (5,6). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (7).

The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (8). DNA damage induces phosphorylation of p53 at Ser15 and Ser20 and leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2 (9). MDM2 inhibits p53 accumulation by targeting it for ubiquitination and proteasomal degradation (10,11).

Stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK) are members of the MAPK family that are activated by a variety of environmental stresses, inflammatory cytokines, growth factors, and GPCR agonists. Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (12). 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 (12,13). c-Jun is a member of the Jun Family, containing c-Jun, JunB, and JunD, and is a component of the transcription factor AP-1 (activator protein-1). Extracellular signals from growth factors, chemokines, and stress activate AP-1-dependent transcription. The transcriptional activity of c-Jun is regulated by phosphorylation at Ser63 and Ser73 through SAPK/JNK (reviewed in 14). AP-1 regulated genes exert diverse biological functions including cell proliferation, differentiation, and apoptosis, as well as transformation, invasion and metastasis, depending on cell type and context (13, 15-17).

p38 MAP kinase (MAPK), also called RK (18) or CSBP (19), is the mammalian orthologue of the yeast HOG kinase that participates in a signaling cascade controlling cellular responses to cytokines and stress (17-20). MKK3, MKK6, and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. MAPKAPK-2 is a direct target of p38 MAPK (17). Multiple residues of MAPKAPK-2 are phosphorylated *in vivo* in response to stress. However, only four residues (Thr25, Thr222, Ser272, and Thr334) are phosphorylated by p38 MAPK in an *in vitro* kinase assay (21). Phosphorylation at Thr222, Ser272, and Thr334 appears to be essential for the activity of MAPKAPK-2 (6). Heat shock protein (HSP) 27 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. HSP27 is phosphorylated at Ser15, Ser78, and Ser82 by MAPKAPK-2 as a result of the activation of the p38 MAP kinase pathway (19,22).

Specificity/Sensitivity: Each antibody in the Stress and Apoptosis Antibody Sampler Kit detects endogenous levels of target protein. Antibodies do not cross-react with any isoforms or phosphorylation sites of the target protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to Asp175 of human Caspase-3 or residues surrounding Asp214 of human PARP. Phospho-specific monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser82 of human HSP27, Ser73 of human c-Jun, Thr334 of human MAPKAPK-2, Ser15 of human p53, Thr180/Tyr182 of human p38 MAPK, or Thr183/Tyr185 of human SAPK/JNK.

U.S. Patent No. 5,675,063

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.

Background References:

(1) Fernandes-Alnemri, T. et al. (1994) *J Biol Chem* 269, 30761-4.

(2) Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-8.

(3) Lazebnik, Y.A. et al. (1994) *Nature* 371, 346-7.

(4) Cohen, G.M. (1997) *Biochem J* 326 (Pt 1), 1-16.

(5) Nicholson, D.W. et al. (1995) *Nature* 376, 37-43.

(6) Tewari, M. et al. (1995) *Cell* 81, 801-9.

(7) Oliver, F.J. et al. (1998) *J Biol Chem* 273, 33533-9.

(8) Levine, A.J. (1997) *Cell* 88, 323-31.

(9) Shieh, S.Y. et al. (1997) *Cell* 91, 325-34.

(10) Chehab, N.H. et al. (1999) *Proc Natl Acad Sci U S A* 96, 13777-82.

(11) Honda, R. et al. (1997) *FEBS Lett* 420, 25-7.

(12) Kyriakis, J.M. and Avruch, J. (2001) *Physiol Rev* 81, 807-69.

(13) Leppä, S. and Bohmann, D. (1999) *Oncogene* 18, 6158-62.

(14) Davis, R.J. (2000) *Cell* 103, 239-52.

(15) Shaulian, E. and Karin, M. (2002) *Nat Cell Biol* 4, E131-6.

(16) Weiss, C. and Bohmann, D. (2004) *Cell Cycle* 3, 111-3.

(17) Rouse, J. et al. (1994) *Cell* 78, 1027-37.

(18) Han, J. et al. (1994) *Science* 265, 808-11.

(19) Lee, J.C. et al. *Nature* 372, 739-46.

(20) Freshney, N.W. et al. (1994) *Cell* 78, 1039-49.

(21) Ben-Levy, R. et al. (1995) *EMBO J* 14, 5920-30.

(22) Landry, J. et al. (1992) *J Biol Chem* 267, 794-803.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.