#12742 Store at -20°C

Procaspase Antibody Sampler Kit

☑ 1 Kit
(7 x 40 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
Caspase-3 (8G10) Rabbit mAb	9665	40 µl	17, 19, 35 kDa	Rabbit IgG
Caspase-6 Antibody	9762	40 µl	15, 35 kDa	Rabbit IgG
Caspase-7 (D2Q3L) Rabbit mAb	12827	40 µl	20, 35 kDa	Rabbit IgG
Caspase-8 (1C12) Mouse mAb	9746	40 µl	18, 43, 57 kDa	Mouse IgG1
Caspase-9 (C9) Mouse mAb	9508	40 µl	47, 37, 35 kDa (H) 51, 39, 37 kDa (R, M)	Mouse IgG1
Lamin A/C (4C11) Mouse mAb	4777	40 µl	74 kDa (Lamin A) 63 kDa (Lamin C)	Mouse IgG2a
PARP Antibody	9542	40 µl	89, 116 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 companion 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
Lamin A/C (4C11) Mouse mAb #4777	
Western blotting:	1:2000

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

Description: The Procaspase Antibody Sampler Kit provides an economical means to evaluate the abundance and activation of caspases. The kit contains enough primary antibody to perform at least four western blots per primary antibody.

Background: Apoptosis is a regulated physiological process leading to cell death. Caspases, a family of cysteine acid proteases, are central regulators of apoptosis. Initiator caspases (including 2, 8, 9, 10 and 12) are closely coupled to proapoptotic signals, which include the FasL, TNF-α, and DNA damage. Once activated, these caspases cleave and activate downstream effector caspases (including 3, 6 and 7), which in turn cleave cytoskeletal and nuclear proteins like PARP, α-fodrin, DFF and lamin A, and induce apoptosis (1,2).

Caspase-8 (FLICE, Mch5, MACH) and Caspase-9 (ICE-LAP6, Mch6) are initiator caspases. CD95 receptor (Fas/APO-1) and tumor necrosis factor receptor 1 (TNFR1) activate caspase-8, leading to the release of the caspase-8 active fragments, p18 and p10 (3-6). Cytochrome c released from the mitochondria associates with procaspase-9 (47 kDa)/Apaf 1. Apaf-1 mediated activation of caspase-9 involves intrinsic proteolytic processing resulting in cleavage at Asp315 and producing a p35 subunit. Another cleavage occurs at Asp330 producing a p37 subunit that can serve to amplify the apoptotic response (7-11).

Caspase-3 (CPP-32, Apoptain, Yama, SCA-1), Caspase-6 (Mch2), and Caspase-7 (CMH-1, Mch3, ICE-LAP3) are effector caspases (12-16). Activation of caspase-3 requires proteolytic processing of its inactive zymogen/proform into activated p17 and p12 subunits (17). Procaspase-7 is activated through proteolytic processing by upstream caspases at Asp23, Asp198, and Asp206 to produce the mature subunits (14,16). Procaspase-6 is cleaved by caspase-3 at Asp23, Asp179 and Asp193 to form active large (p18) and small (p11) subunits (7).

PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, appears to be involved in DNA repair in response to environmental stress (18). This protein can be cleaved by many ICE-like caspases *in vitro* (2,19) and is one of the main cleavage targets of caspase-3 *in vivo* (17,20). In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (17,19). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (21).

Lamins are nuclear membrane structural components that are important in maintaining normal cell functions such as cell cycle control, DNA replication, and chromatin organization (22-24). Lamin A/C is cleaved by caspase-6 and serves as a marker for caspase-6 activation. During apoptosis, lamin A/C is specifically cleaved into large (41-50 kDa) and small (28 kDa) fragments (24,25). The cleavage of lamins results in nuclear disregulation and cell death (26,27).

Specificity/Sensitivity: Each antibody in the Procaspase Antibody Sampler Kit detects endogenous levels of its respective target. Caspase-3 (8G10) Rabbit mAb detects full-length (35 kDa) and the large fragment (17/19 kDa) of caspase-3 resulting from cleavage at Asp175. Caspase-6 Antibody detects both full length caspase-6 (35 kDa) and the small subunit (15 kDa) of caspase-6 resulting from cleavage at Asp193. Caspase-7 (D2Q3L) Rabbit mAb detects both the full-length (35 kDa) and the large subunit (20 kDa) of caspase-7 resulting from cleavage at Asp198. Caspase-8 (1C12) Mouse mAb detects full length (57 kDa), the cleaved intermediate p43/p41, and the p18 fragment of caspase-8. Caspase-9 (C9) Antibody detects full-length caspase-9, as well as the large fragments resulting from cleavage at Asp315 and Asp330. PARP Antibody detects full length PARP1 (116 kDa), as well as the large fragment (89 kDa) of PARP1 resulting from caspase cleavage at Asp214. Lamin A/C (4C11) Mouse mAb detects full-length lamin A and lamin C proteins, as well as the larger fragments of lamin A (50 kDa) and lamin C (41 kDa) resulting from caspase cleavage. Caspase-9 (C9) Antibody detects endogenous levels of the pro form of caspase-9 as well as cleaved fragments.

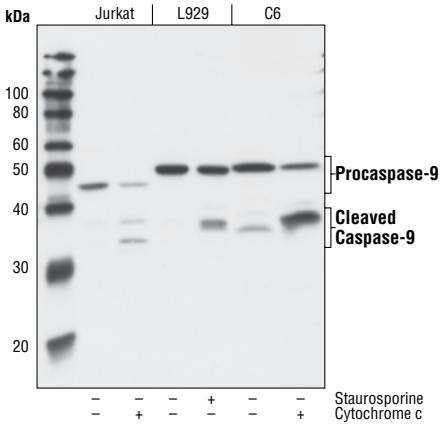
Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to (Asp175) in human caspase-3 protein, residues surrounding Pro158 of human caspase-7 protein, the carboxy-terminal sequence of the p18 fragment of human caspase-8 protein, recombinant human caspase-9 protein or human lamin A protein.

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the cleavage site of caspase-6 or the caspase cleavage site in PARP. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

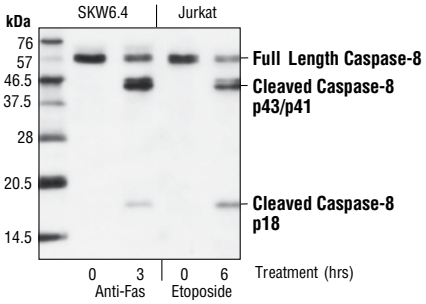
U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

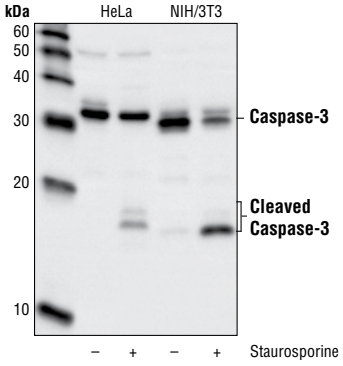
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



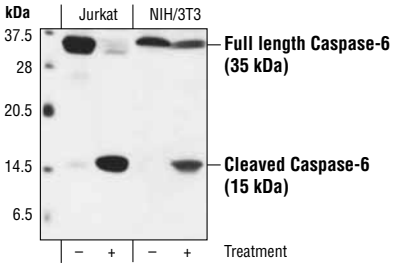
Western blot analysis of extracts from Jurkat, L929, and C6 cells untreated (-) or treated with Staurosporine #9953 or cytochrome c as indicated, using ***Caspase 9 (C9) Mouse mAb #9508****.*



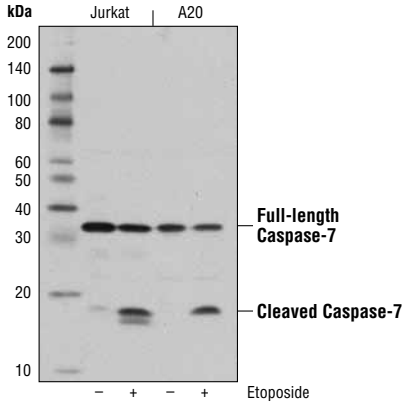
Western blot analysis of extracts from SKW6.4 cells, untreated or anti-Fas-treated (1 µg/ml), and Jurkat cells, untreated or treated with Etoposide #2200 (25 µM), using ***Caspase-8 (1C12) Mouse mAb #9746****.*



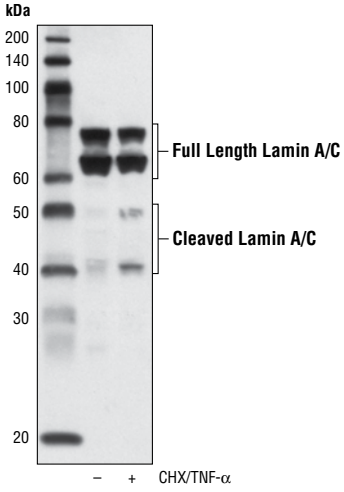
Western analysis of extracts from HeLa and NIH/3T3 cells, untreated (-) or treated with Staurosporine #9953 (1 µM, 3 hr; +), using ***Caspase-3 (8G10) Rabbit mAb #9665****.*



Western blot analysis of extracts from NIH/3T3 cells, untreated (-) or treated with Staurosporine #9953 (1 µM; +) and Jurkat cells, untreated (-) or treated with Etoposide #2200 (25 µM; +), using ***Caspase-6 Antibody #9762****.*



Western blot analysis of extracts from Jurkat and A20 cells, untreated (-) or treated with Etoposide #2200 (25 µM, overnight; +), using ***Caspase-7 (D2Q3L) Rabbit mAb #12827****.*



Western blot analysis of extracts from THP-1 cells, untreated (-) or cycloheximide-treated (CHX, 10 µg/ml, overnight; +) followed by treatment with Human Tumor Necrosis Factor-α (hTNF-α) #8902 (20 ng/ml, 4 hr; +), using ***Lamin A/C (4C11) Mouse mAb #4777.***

Background References:

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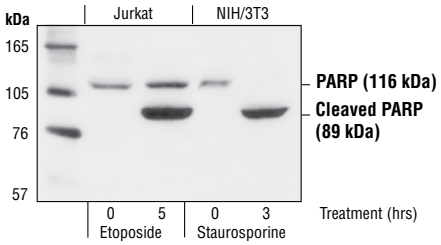
(27) Rao, L. et al. (1996) *J Cell Biol* 135, 1441-55.



Western blot analysis of extracts from NIH/3T3 cells, untreated (-) or treated with Staurosporine #9953 (1 µM; +), and Jurkat cells, untreated (-) or treated with Etoposide #2200 (25 µM; +), using ***PARP Antibody #9542****.*

#12742

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.