

Apoptosis Antibody Sampler Kit



✓ 1 Kit
 (8 x 40 microliters)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb	9664	40 µl	17, 19 kDa	Rabbit IgG
Caspase-3 Antibody	9662	40 µl	17, 19, 35 kDa	Rabbit IgG
PARP Antibody	9542	40 µl	24, 89, 116 kDa	Rabbit IgG
Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb	5625	40 µl	89 kDa	Rabbit IgG
Caspase-9 Antibody (Human Specific)	9502	40 µl	17, 35, 37, 47 kDa	Rabbit IgG
Cleaved Caspase-9 (Asp330) Antibody (Human Specific)	9501	40 µl	17, 37 kDa	Rabbit IgG
Caspase-7 Antibody	9492	40 µl	20, 35 kDa	Rabbit IgG
Cleaved Caspase-7 (Asp198) Antibody	9491	40 µl	20 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The Apoptosis Antibody Sampler Kit provides an economical means to evaluate the levels of inactive and active caspases. The kit contains enough primary and secondary antibodies to perform four Western blot experiments with each 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 8, 9, 10 and 12) are closely coupled to proapoptotic signals. 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. Cytochrome c released from mitochondria is coupled to the activation of caspase-9, a key initiator caspase (1). Proapoptotic stimuli include the FasL, TNF- α , DNA damage and ER stress. Fas and TNFR activate caspases 8 and 10 (2), DNA damage leads to the activation of caspase-9 and ER stress leads to the calcium-mediated activation of caspase-12 (3). The inhibitor of apoptosis protein (IAP) family includes XIAP and survivin and functions by binding and inhibiting several caspases (4,5). Smac/Diablo, a mitochondrial protein, is released into the cytosol upon mitochondrial stress and competes with caspases for binding of IAPs. The interaction of Smac/Diablo with IAPs relieves the inhibitory effects of the IAPs on caspases (6).

Specificity/Sensitivity: Each antibody in the Apoptosis Antibody Sampler Kit detects endogenous levels of its respective protein. Cleaved Caspase-3 (Asp175), Cleaved Caspase-7 (Asp198) and Cleaved PARP (Asp214) Antibodies recognize only the large fragments of their respective cleaved proteins. Cleaved Caspase-9 (Asp330) Antibody recognizes either the 37 kDa (with prodomain) or 17 kDa large fragment of the cleaved protein. Caspase-3, Caspase-7, Caspase-9 and PARP Antibodies recognize both the full length and large cleaved fragments of their respective proteins.

Source/Purification: Monoclonal and polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding the proteolytic cleavage sites of human caspase-3, -7 and -9 and human PARP. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

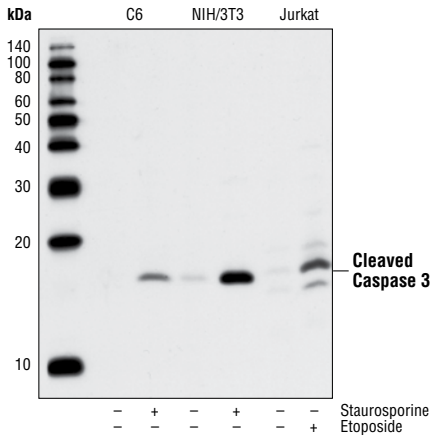
- (1) Baker, S.J. and Reddy, E.P. (1998) *Oncogene* 17, 3261–3270.
- (2) Budihardjo, I. et al. (1999) *Annu. Rev. Cell Dev. Biol.* 15, 269–290.
- (3) Nakagawa, T. et al. (2000) *Nature* 403, 98–103.
- (4) Deveraux, Q.L. et al. (1998) *EMBO J.* 17, 2215–2223.
- (5) Li, F. et al. (1998) *Nature* 396, 580–584.
- (6) Du, C. et al. (2000) *Cell* 102, 33–42.

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

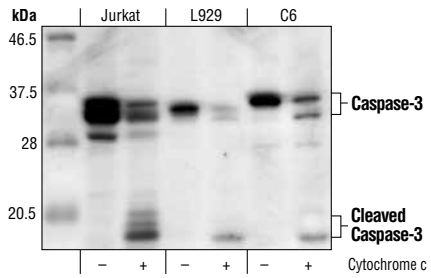
Recommended Antibody Dilutions:

Western blotting 1:1000

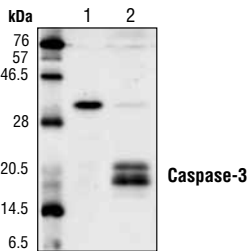
Please visit www.cellsignal.com for a complete listing of recommended companion products.



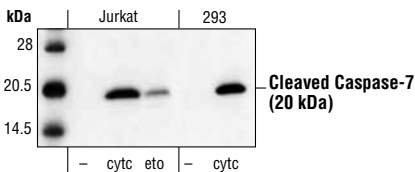
Western blot analysis of extracts from C6 (rat), NIH/3T3 (mouse), and Jurkat (human) cells, untreated or treated with staurosporine or etoposide as indicated, using **Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664**.



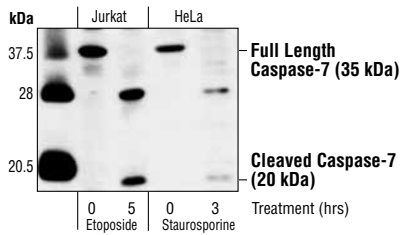
Western blot analysis of extracts from Jurkat, L929 and C6 cells, untreated or treated with 0.25 mg/ml cytochrome c, using **Caspase-3 Antibody #9662**.



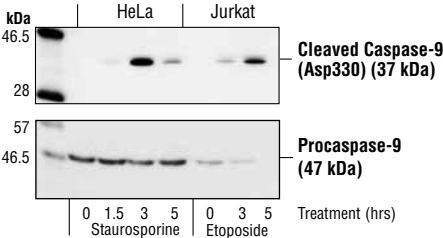
Western blot analysis of extracts from Jurkat cells, untreated or treated with cytochrome c, showing full length and cleaved caspase-3 and cleaved caspase-3 (19 and 17 kDa) fragments, using **Caspase-3 Antibody #9662**.



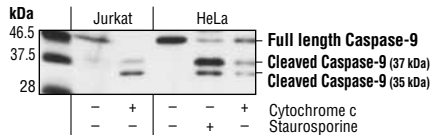
Western blot analysis of extracts from Jurkat cells and 293 cells treated with 0.25 mg/ml cytochrome c for 1 hour (cytc) or with 25 μ M etoposide for 5 hours (eto), using **Cleaved Caspase-7 (Asp198) Antibody #9491**.



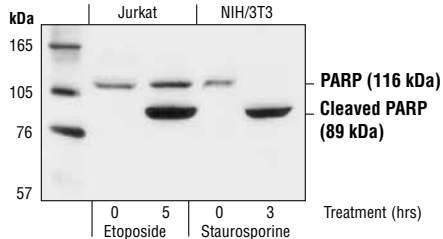
Western blot analysis of extracts from Jurkat cells, untreated or etoposide-treated (25 μ M), and HeLa cells, untreated or staurosporine-treated (1 μ M), using **Caspase-7 Antibody #9492**.



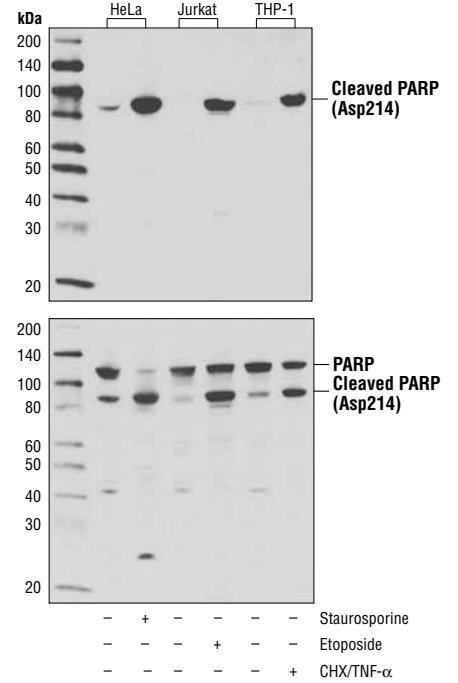
Western blot analysis of extracts from HeLa cells, untreated or staurosporine-treated (1 μ M), and Jurkat cells, untreated or etoposide-treated, using **Cleaved Caspase-9 (Asp330) Antibody (Human Specific) #9501** (upper) or full length Caspase-9 Antibody #9502 (lower).



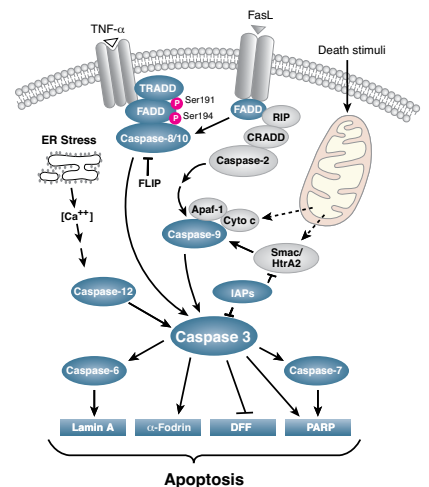
Western blot analysis of extracts from HeLa cells, untreated, staurosporine-treated (1 μ M) or cytochrome c-treated (0.25 mg/ml), and Jurkat cells, untreated or cytochrome c-treated, using **Caspase-9 Antibody (Human Specific) #9502**.



Western blot analysis of extracts from NIH/3T3 cells, untreated or staurosporine-treated (1 μ M), and Jurkat cells, untreated or etoposide-treated (25 μ M), using **PARP Antibody #9542**.



Western blot analysis of extracts from HeLa cells, untreated or treated with Staurosporine #9953 (1 μ M, 3 hr), Jurkat cells, untreated or etoposide-treated (25 μ M, overnight), and THP-1 cells, untreated or cycloheximide-treated (CHX, 10 μ g/ml, overnight) followed by treatment with TNF- α #8902 (20 ng/ml, 4 hr), using **Cleaved PARP (Asp214) (D64E10) XP[®] Rabbit mAb #5625** (upper), or total PARP Antibody #9542 (lower).



Apoptosis/ Caspase Signaling Pathway

Western Immunoblotting Protocol (Primary Ab Incubation In Milk)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.