

Apoptosis Antibody Sampler Kit



1 Kit
(8 x 20 μl)

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rev. 03/04/19

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
Caspase-3 (D3R6Y) Rabbit mAb	14220	20 μl	17, 19, 35 kDa	Rabbit IgG
PARP Antibody	9542	20 μl	24, 89, 116 kDa	Rabbit IgG
Cleaved PARP (Asp214) (D64E10) XP [®] Rabbit mAb	5625	20 μl	89 kDa	Rabbit IgG
Caspase-9 (C9) Mouse mAb	9508	20 μl	47, 37, 35 kDa (H) 51, 39, 37 kDa (R,M)	Mouse IgG1
Cleaved Caspase-9 (Asp330) (E5Z7N) Rabbit mAb	52873	20 μl	37 kDa	Rabbit IgG
Caspase-7 (D2Q3L) Rabbit mAb	12827	20 μl	20, 35 kDa	Rabbit IgG
Cleaved Caspase-7 (Asp198) (D6H1) Rabbit mAb	8438	20 μl	18 kDa	Rabbit IgG
Anti-mouse IgG, HRP-linked Antibody	7076	100 μl		Horse
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 two 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 its respective target at endogenous levels. Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb, Cleaved Caspase-7 (Asp198) (D6H1) Rabbit mAb, Cleaved Caspase-9 (Asp330) (E5Z7N) Rabbit mAb, and Cleaved PARP (Asp214) (D64E10) XP[®] Rabbit mAb detect only the large cleaved fragments of their respective targets. Caspase-3 (D3R6Y) Rabbit mAb, Caspase-7 (D2Q3L) Rabbit mAb, Caspase-9 (C9) Mouse mAb, and PARP Antibody detect both the full length and the large cleaved fragments of their respective targets.

Source/Purification: Monoclonal and polyclonal antibodies are produced by immunizing animals with recombinant human caspase-9 and the proteolytic cleavage sites of human caspase-3 protein, or with synthetic peptides corresponding to residues surrounding Pro158 of human caspase-7 protein, or with the proteolytic cleavage sites of human caspase-9 and PARP proteins. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Storage:

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

Recommended Antibody Dilutions:

Western blotting 1:1000

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

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.

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

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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 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 dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 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 dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

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

II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- 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.
- 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.