#12675 Store at -20°C

Initiator Caspases Antibody Sampler Kit

1 Kit
(5 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Caspase-2 (C2) Mouse mAb	2224	20 µl	12, 14, 48 kDa	Mouse IgG1
Caspase-3 (D3R6Y) Rabbit mAb	14220	20 µl	17, 19, 35 kDa	Rabbit IgG
Caspase-8 (1C12) Mouse mAb	9746	20 µl	18, 43, 57 kDa	Mouse IgG1
Cleaved Caspase-9 (Asp330) (E5Z7N) Rabbit mAb	52873	20 µl	37 kDa	Rabbit IgG
Caspase-9 (C9) Mouse mAb	9508	20 µl	47, 37, 35 kDa (H) 51, 39, 37 kDa (R, M)	Mouse IgG1
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 antibody.*

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 Initiator Caspases Antibody Sampler Kit provides an economical means of evaluating initiator (apical) caspase proteins. The kit contains enough primary antibody to perform two western blots with each 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 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 such as PARP, α-fodrin, DFF and lamin A; inducing apoptosis (1,2).

Formation of a death-inducing signaling complex (DISC) around the receptors for death factors, including FasL and TNF-α, is essential for receptor-mediated apoptosis (3). Upon ligand activation, Fas and TNF-R1 associate with death domain (DD) containing adaptor proteins FADD (Fas associated death domain) (4,5) and TRADD (TNF-R1 associated death domain) (6). In addition to a carboxy-terminal DD, FADD contains an amino-terminal death effector domain (DED) that binds to DEDs and activates initiator caspase 8 (FLICE, Mch5, MACH) and caspase 10 (FLICE2, Mch4) (7-12). TRADD does not contain a DED and therefore must associate with FADD in response to TNF-R1 driven apoptosis (13).

Caspase-9 (ICE-LAP6, Mch6) is activated through the mitochondrial-mediated pathway. Cytochrome c released from mitochondria associates with procaspase-9 (47 kDa)/Apaf-1. Apaf-1 mediated activation of caspase-9 involves proteolytic processing resulting in cleavage at Asp315 and producing a p35 subunit. Another cleavage occurs at Asp330 producing a p37 subunit that can amplify the apoptotic response (14-17).

Caspase-2 (Nedd2/ICH-1) is the nuclear apoptotic respondent to cellular genotoxic stress or mitotic catastrophe. The procaspase is cleaved at Asp316, producing a 14 kDa fragment and a 32 kDa prodomain/large subunit. Subsequent processing at Asp152 and Asp330 produces an 18 kDa large subunit and a 12 kDa small fragment (18). Activation occurs upon recruitment to a complex containing a p53-induced death domain protein, PIDD (19). This suggests that caspase-2 can be a nuclear initiator caspase with a requirement for caspase-9 and caspase-3 activation in downstream apoptotic events (20,22). In apoptotic pathways resulting from UV-induced DNA damage, processing of caspase-2 occurs downstream of mitochondrial dysfunction and of caspase-9 and caspase-3 activation, extending a possible role for caspase-2 as a parallel effector caspase (22).

Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis and caspase-3 cleavage is a key indicator of initiator caspase activation. Caspase-3 is either partially or totally responsible for the proteolytic cleavage of many key proteins including the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (23). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments (24).

Specificity/Sensitivity: Each antibody in the Initiator Caspases Antibody Sampler Kit recognizes its respective target at endogenous levels. Caspase-3 (D3R6Y) Rabbit mAb detects full-length caspase-3 (35 kDa) as well as the large subunit (p20) of caspase-3 resulting from cleavage during apoptosis. Caspase-8 (1C12) Mouse mAb recognizes full length (57 kDa), the cleaved intermediate p43/p41, and the p18 fragment of caspase-8. Caspase-9 (C9) Antibody recognizes full-length caspase-9, as well as the large fragments resulting from cleavage at Asp315 and Asp330. Cleaved Caspase-9 (Asp330) (E5Z7N) Rabbit mAb recognizes caspase-9 protein only when cleaved at Asp330. Caspase-2 (C2) Mouse mAb recognizes procaspase-2, as well as its 14 and 12 kDa cleaved fragments.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a recombinant protein specific to either the p20 subunit of human caspase-3 protein, or human caspase-9 protein. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp330 of human caspase-9, the carboxy-terminal sequence of the p18 fragment of human caspase-8, or the carboxy-terminal portion of human caspase-2.

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 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.

Background References:

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