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#92570

Apoptosis/Necroptosis Antibody Sampler Kit

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Entrez-Gene ID #836, 841, 197259, 8737
UniProt ID #P42574, Q14790, Q8NB16, Q13546

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
P-RIP (S166) (D1L3S) Rabbit mAb	65746	20 µl	78-82 kDa	Rabbit IgG
RIP (D94C12) XP® Rabbit mAb	3493	20 µl	78 kDa	Rabbit IgG
P-MLKL (S358) (D6H3V)Rabbit mAb	91689	20 µl	54 kDa	Rabbit IgG
MLKL (D2I6N) Rabbit mAb	14993	20 µl	54 kDa	Rabbit IgG
Cleaved Caspase-3 (D175) (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
Cleaved Caspase-8 (Asp384) (11G10) Mouse mAb	9748	20 µl	10 kDa	Mouse IgG1
Caspase-8 (D35G2) Rabbit mAb	4790	20 µl	10, 57 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

Description: The Apoptosis/Necroptosis Antibody Sampler Kit provides an economical means of detecting markers for apoptosis and necroptosis. The kit contains enough primary antibody to perform at least two western blot experiments.

Background: Apoptosis is a regulated physiological process leading to cell death (1,2). Caspases, a family of cysteine acid proteases, are central regulators of apoptosis. Caspases are synthesized as inactive zymogens containing a pro-domain followed by large (p20) and small subunits (p10) that are proteolytically processed in a cascade of caspase activity. 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. Apoptosis induced through the extrinsic mechanisms involving death receptors in the tumor necrosis factor receptor superfamily activates caspase-8. Activated caspase-8 cleaves and activates downstream effector caspases, such as caspase-1, -3, -6, and -7. Caspase-3 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).

Necroptosis, a regulated pathway for necrotic cell death, is triggered by a number of inflammatory signals, including cytokines in the tumor necrosis factor (TNF) family, pathogen sensors such as toll-like receptors (TLRs), and ischemic injury (3,4). Necroptosis is negatively regulated by caspase-8 mediated apoptosis in which the kinase RIP/RIPK1 is cleaved (5). Furthermore, necroptosis is inhibited by a small molecule inhibitor of RIP, necrostatin-1 (Nec-1) (6). Research studies show that necroptosis contributes to a number of pathological conditions, and Nec-1 has been shown to provide neuroprotection in models such as ischemic brain injury (7). RIP is phosphorylated at several sites within the kinase domain that are sensitive to Nec-1, including Ser14, Ser15, Ser161, and Ser166 (8). Phosphorylation drives association with RIP3, which is required for necroptosis (9-11). Mixed lineage kinase domain-like protein (MLKL) is a pseudokinase that was identified as downstream target of RIP3 in the necroptosis pathway (12). During necroptosis RIP3 is phosphorylated at Ser227, which recruits MLKL and leads to its phosphorylation at Thr357 and Ser358 (12). Knockdown of MLKL through multiple mechanisms results in inhibition of necroptosis (13). While the precise mechanism for MLKL-induced necroptosis is unclear, some studies have shown that necroptosis leads to oligomerization of MLKL and translocation to the plasma membrane, where it effects membrane integrity (14-17).

Specificity/Sensitivity: Each antibody in the Apoptosis/Necroptosis Antibody Sampler Kit detects endogenous levels of its target protein. MLKL (D2I6N) Rabbit mAb cross-reacts with a band at 130 kDa in some cell lines. Phospho-MLKL (Ser358) (D6H3V) Rabbit mAb may also react with MLKL when dually phoshorylated at Thr357 and Ser358. Caspase-3 (D3R6Y) Rabbit mAb detects full-length caspase-3 as well as the large subunit (p20) of caspase-3 resulting from cleavage during apoptosis. Cleaved Caspase-3 (Asp175) (5A1) Rabbit mAb detects endogenous levels of the large subunit of caspase-3 but does not detect full-length caspase-3 or other cleaved caspases. Cleaved Caspase-8 (Asp384) (11G10) Mouse mAb detects endogenous levels of the small fragment of caspase-8 resulting from cleavage at aspartic acid 384. The antibody does not cross-react with full length caspase-8. Caspase-8 (D35G2) Rabbit mAb detects endogenous levels of total caspase-8, including the p10 subunit of the activated protein. It may also cross-react with overexpressed levels of caspase-10.

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

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

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(6) Degterev, A. et al. (2008) *Nat Chem Biol* 4, 313-21.
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(12) Sun, L. et al. (2012) *Cell* 148, 213-27.
(13) Wu, J. et al. (2013) *Cell Res* 23, 994-1006.
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Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides surrounding Leu190 of human RIP1, the carboxy terminus of human MLKL and human caspase-8, the amino-terminal sequence of p10 of human caspase-8, amino terminal residues adjacent to Asp175 of human caspase-3, a recombinant protein corresponding to the p20 subunit of caspase-3, and phosphopeptides surrounding human Ser166 of human RIP and Ser358 of human MLKL.

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