Store at -20°C

#48734

Mouse Reactive Cell Death and Autophagy Antibody Sampler Kit

Support: +1-978-867-2388 (U.S.)
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New 06/20

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/ Source
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb	9664	20 µl	17, 19 kDa	Rabbit IgG
Cleaved PARP (Asp214) (D6X6X) Rabbit mAb (Rodent Specific)	94885	20 µl	89 kDa	Rabbit IgG
Phospho-RIP (Ser166) (E7G6O) Rabbit mAb	53286	20 µl	78 kDa	Rabbit IgG
Phospho-RIP3 (Thr231/Ser232) (E7S1R) Rabbit mAb	91702	20 µl	46-62 kDa	Rabbit IgG
Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb	37333	20 µl	54 kDa	Rabbit IgG
Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb	10137	20 µl	31 kDa	Rabbit IgG
Cleaved-IL-1β (Asp117) (E7V2A) Rabbit mAb (Mouse Specific)	63124	20 µl	17 kDa	Rabbit IgG
LC3B (E5Q2K) Mouse mAb	83506	20 µl	14, 16 kDa	Mouse IgG2b
SQSTM1/p62 (D6M5X) Rabbit mAb (Rodent Specific)	23214	20 µl	62 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.

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.*

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

Background References:

(1) Galluzzi, L. et al. (2018) *Cell Death Differ* 25, 486-541.

(2) Green, D.R. (1998) *Cell* 94, 695-8.

(3) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ* 12 Suppl 2, 1509-18.

(4) Shan, B. et al. (2018) *Genes Dev* 32, 327-40.

(5) Shi, J. et al. (2017) *Trends Biochem Sci* 42, 245-54.

Description: The Mouse Reactive Cell Death and Autophagy Antibody Sampler Kit provides an economical means of detecting common readouts in apoptosis, necroptosis, pyroptosis, and autophagy. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: Regulated cell death has been classified based on distinct morphological and biochemical pathways (1). Type I cell death, or apoptosis, is characterized by cytoplasmic shrinkage, chromatin condensation, nuclear fragmentation, plasma membrane blebbing, and phagocytic update of dead cells. Apoptosis can occur through extrinsic pathways involving extracellular factors, including the activation of death receptors, or through intrinsic pathways involving intracellular perturbations, including mitochondrial outer membrane permeabilization (2). Both of these apoptotic pathways lead to activation of caspases, a family of cysteine acid proteases that are synthesized as inactive zymogens containing pro-domains, followed by large (p20) and small (p10) subunits which are proteolytically activated in a cascade-like fashion. Caspase-3 is a key downstream protease activated by both extrinsic and intrinsic apoptotic pathways and cleaves a large number of proteins involved in the disassembly of the cell, including poly(ADP-ribose) polymerase (PARP), a protein involved in the DNA damage response.

Type II cell death, or autophagy, manifests with extensive cytoplasmic vacuolization, and like apoptosis, can include phagocytic update. Autophagy is a catabolic process for the degradation of cellular components including protein aggregates, damaged organelles, and pathogens (3). The process involves the engulfment of these components into a double membrane structure, the autophagosome, which fuses to the lysosome for degradation. Autophagy requires, and can be monitored by, the conversion of LC3 family members, such as LC3B, from a type I form to a lipidated type II form that is incorporated into the autophagosome membrane and binds to a variety of cargo receptors. Cargo receptors such as SQSTM1/p62 bind LC3 along with ubiquitinated proteins that are targeted for degradation. SQSTM1/p62 is also degraded during this process, and thus its expression is frequently used to monitor this process.

Type III cell death, or necrosis, manifests with plasma membrane permeability with cellular swelling and fragmentation, and lacks a clear phagocytic response which then leads to an inflammatory signaling with the release of damage-associated molecular patterns (DAMPs). Necrosis can be triggered by multiple regulated pathways including necroptosis and pyroptosis. Necroptosis is regulated by the kinase activities of RIP and RIP3 and the pore forming ability of MLKL (4). Necroptosis requires the activation of RIP3 which then phosphorylates MLKL at Ser358 (Ser345 in mouse). Phosphorylation of MLKL leads to generation of a pore complex involved in cell swelling and the secretion of DAMPs. RIP3 activation is triggered through several RIP homotypic interaction motif (RHIM) domain interactions including RIP, TRIF, and ZBP1 and results in the phosphorylation of RIP3 at Ser227 (Thr231/Ser232 in mouse). Canonical necroptosis signaling is mediated by RIP, and this can be inhibited by necrostatins, small molecules that directly inhibit RIP kinase activity. Activation of RIP can be monitored through autophosphorylation sites including Ser166. Pyroptosis is generally induced in cells of the innate immune system, and is characterized by cleavage of Gasdermin D (5). The amino-terminal fragment of Gasdermin D produced following cleavage by inflammatory caspases (Caspase-1, -4, -5), oligomerizes to form a pore. Canonical cleavage of Gasdermin D occurs through a two-step process. The first step involves transcriptional regulation of targets such as NLRP3 and the pro-forms of IL-1β and IL-18. In the second execution step, Caspase-1 is activated through formation of inflammasome complexes. Activated Caspase-1 cleaves Gasdermin D as well as IL-1β and IL-18 to their mature forms, and these active cytokines are secreted through pores formed by Gasdermin D.

Specificity/Sensitivity: Each antibody in the Mouse Reactive Cell Death and Autophagy Antibody Sampler Kit detects endogenous levels of its target protein. Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full-length caspase-3 or other cleaved caspases. Cleaved PARP (Asp214) (D6X6X) Rabbit mAb (Rodent Specific) recognizes endogenous levels of the large fragment (89 kDa) of rodent PARP only when cleaved at Asp214. Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb recognizes endogenous levels of the amino fragment of mouse Gasdermin D protein only when cleaved at Asp276. Cleaved-IL-1β (Asp117) (E7V2A) Rabbit mAb (Mouse Specific) recognizes endogenous levels of mouse IL-1β protein only when cleaved at Asp117. Phospho-RIP3 (Thr231/Ser232) (E7S1R) Rabbit mAb recognizes endogenous levels of RIP3 protein only when phosphorylated at Thr231/Ser232. This antibody may not recognize RIP3 when only singly phosphorylated at Thr231 or Ser232. Phospho-RIP (Ser166) (E7G6O) Rabbit mAb recognizes endogenous levels of RIP protein only when phosphorylated at Ser166. Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb recognizes endogenous levels of mouse MLKL protein only when phosphorylated at Ser345. Weak, non-specific nuclear staining has been observed by immunofluorescence (IF-IC). LC3B (E5Q2K) Mouse mAb detects both type I and type II forms of LC3B. Cross reactivity was not detected with other family members. SQSTM1/p62 (D6M5X) Rabbit mAb (Rodent Specific) recognizes endogenous levels of total rodent SQSTM1/p62 protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Asp175 of human caspase-3, Asp214 of rodent PARP, Asp276 of mouse Gasdermin D, Asp117 of mouse IL-1β, Gly300 of mouse SQSTM1/p62, residues near the amino terminus of human LC3B, and synthetic phosphopeptides corresponding to Ser166 of mouse RIP, Thr231/Ser232 of mouse RIP3, and Ser345 of mouse 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.