Revision 1

Store at -20C

#42867

Human Reactive Cell Death and Autophagy Antibody Sampler Kit

1 Kit (9 x 20 microliters)

Cell Signaling TECHNOLOGY®

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Product Includes	**Product #**	**Quantity**	**Mol. Wt**	**Isotype/Source**
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb	9664	20 µl	17, 19 kDa	Rabbit IgG
Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb	5625	20 µl	89 kDa	Rabbit IgG
Phospho-RIP (Ser166) (D1L3S) Rabbit mAb	65746	20 µl	78-82 kDa	Rabbit IgG
Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb	93654	20 µl	46-62 kDa	Rabbit IgG
Phospho-MLKL (Ser358) (D6H3V) Rabbit mAb	91689	20 µl	54 kDa	Rabbit IgG
Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb	36425	20 µl	30 kDa	Rabbit IgG
Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb	83186	20 µl	17 kDa	Rabbit IgG
LC3B (E5Q2K) Mouse mAb	83506	20 µl	14, 16 kDa	Mouse IgG2b
SQSTM1/p62 (D5L7G) Mouse mAb	88588	20 µl	62 kDa	Mouse IgG1
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Human 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.

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.*

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.

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.

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