Store at -20°C

#47928

Mouse Reactive Necroptosis Antibody Sampler Kit

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New 05/20

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Phospho-RIP (Ser166) (E7G6O) Rabbit mAb	53286	20 µl	78 kDa	Rabbit IgG
RIP (D94C12) XP® Rabbit mAb	3493	20 µl	78 kDa	Rabbit IgG
Phospho-RIP3 (Thr231/Ser232) (E7S1R) Rabbit mAb	91702	20 µl	46-62 kDa	Rabbit IgG
RIP3 (D8J3L) Rabbit mAb	15828	20 µl	46-62 kDa	Rabbit IgG
Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb	37333	20 µl	54 kDa	Rabbit IgG
MLKL (D6W1K) Rabbit mAb (Mouse Specific)	37705	20 µl	54 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.

Description: The Mouse Reactive Necroptosis Antibody Sampler Kit provides an economical means of detecting total and phosphorylated proteins associated with necroptosis. The kit includes enough antibody to perform two western blots with each primary antibody.

Background: 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), ischemic injury, and neurodegenerative diseases (1-3). The process is negatively regulated by caspases and is initiated through a complex containing the RIP and RIP3 kinases, typically referred to as the necrosome. Necroptosis is inhibited by a small molecule inhibitor of RIP, necrostatin-1 (Nec-1) (4). RIP is phosphorylated at several sites within the kinase domain that are sensitive to Nec-1, including Ser14, Ser15, Ser161, and Ser166 (5). During necroptosis, RIP3 is phosphorylated at Ser227, leading to recruitment and phosphorylation of MLKL at Thr357 and Ser358 (6). Phosphorylation of MLKL results in its oligomerization and translocation to the plasma membrane, where it affects membrane integrity (7-10).

In mice, activation of RIP3 is associated with phosphorylation at Thr231 and Ser232 (11), and then MLKL is phosphorylated at Ser345 by RIP3 (12).

Specificity/Sensitivity: Each antibody in the Mouse Reactive Necroptosis Antibody Sampler Kit detects endogenous levels of its target protein. Phospho-RIP3 (Thr231/Ser232) (E7S1R) Rabbit mAb may not recognize RIP3 when only singly phosphorylated at either Thr231 or Ser232.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to Leu190 of human RIP, His411 of mouse RIP3, and residues near the carboxyl terminus of mouse MLKL protein. Phospho-specific monoclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides corresponding to Ser166 of mouse RIP, Thr231/Ser232 of mouse RIP3, and Ser345 of mouse MLKL protein.

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) Christofferson, D.E. and Yuan, J. (2010) *Curr Opin Cell Biol* 22, 263-8.

(2) Kaczmarek, A. et al. (2013) *Immunity* 38, 209-23.

(3) Zhou, W. and Yuan, J. (2014) *Semin Cell Dev Biol* 35, 14-23.

(4) Degterev, A. et al. (2008) *Nat Chem Biol* 4, 313-21.

(5) Ofengeim, D. and Yuan, J. (2013) *Nat Rev Mol Cell Biol* 14, 727-36.

(6) Sun, L. et al. (2012) *Cell* 148, 213-27.

(7) Cai, Z. et al. (2014) *Nat Cell Biol* 16, 55-65.

(8) Chen, X. et al. (2014) *Cell Res* 24, 105-21.

(9) Wang, H. et al. (2014) *Mol Cell* 54, 133-46.

(10) Dondelinger, Y. et al. (2014) *Cell Rep* 7, 971-81.

(11) Chen, W. et al. (2013) *J Biol Chem* 288, 16247-61.

(12) Murphy, J.M. et al. (2013) *Immunity* 39, 443-53.

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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.