

Store at
-20°C

#98303

Mouse Reactive Pyroptosis Antibody Sampler Kit



Support: +1-978-867-2388 (U.S.)
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New 10/20

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Gasdermin D (E9S1X) Rabbit mAb	39754	20 µl	53, 30 kDa	Rabbit IgG
Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb	10137	20 µl	31 kDa	Rabbit IgG
IL-1β (D3H1Z) Rabbit mAb (Mouse Specific)	12507	20 µl	17, 31 kDa	Rabbit IgG
Cleaved-IL-1β (Asp117) (E7V2A) Rabbit mAb (Mouse Specific)	63124	20 µl	17 kDa	Rabbit IgG
Caspase-1 (E2Z1C) Rabbit mAb	24232	20 µl	48, 10 kDa	Rabbit IgG
Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb	89332	20 µl	22 kDa	Rabbit IgG
Caspase-11 (17D9) Rat mAb	14340	20 µl	38, 43 kDa	Rat IgG2a
ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific)	67824	20 µl	22 kDa	Rabbit IgG
HMGB1 (D3E5) Rabbit mAb	6893	20 µl	29 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 Pyroptosis Antibody Sampler Kit provides an economical means of detecting proteins that are used as readouts for pyroptosis. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: Pyroptosis is a regulated pathway of cell death with morphological features of necrosis, including cell swelling, plasma membrane pore formation, and engagement of an inflammatory response with the release of a number of damage-associated molecular patterns (DAMPs), such as HMGB1 and inflammatory cytokines like IL-1β and IL-18 (1,2). Pyroptosis is generally induced in cells of the innate immune system, such as monocytes, macrophages, and dendritic cells in the presence of pathogen-associated molecular patterns (PAMPs) expressed on microbial pathogens or by cell-derived DAMPs. It is induced through assembly of inflammasomes triggering proteolytic activation of caspase-1 which then cleaves inflammatory cytokines like IL-1β and IL-18 to their mature forms (3). A critical feature of pyroptosis is the cleavage of Gasdermin D by caspase-1 and mouse caspase-11 (or human caspase-4/5) (4-6). Upon cleavage, the N-terminal fragment of Gasdermin D oligomerizes to form a pore, allowing secretion of inflammatory DAMPs and cytokines. Canonical inflammasome assembly typically consists of a cytosolic-pattern recognition receptor (PPR; a nucleotide binding domain and leucine-rich repeat [NLR] or AIM2-like family members), an adaptor protein (ASC/TMS1), and pro-caspase-1. Distinct inflammasome complexes can recognize distinct PAMPs and DAMPs to trigger pyroptosis. The best characterized pathway triggered by the NLR, NLRP3, occurs through a two-step process. The first step is a priming

signal, NF-κB is activated to induce the expression of a number of inflammasome components including NLRP3, pro-IL-1β, and pro-IL-18. In the second activation step, caspase-1 is activated and Gasdermin D and cytokines are proteolytically activated. In a non-canonical pathway, caspase-4 and caspase-5 can directly trigger Gasdermin D cleavage in monocytes following LPS stimulation (5,7).

Specificity/Sensitivity: Gasdermin D (E9S1X) Rabbit mAb recognizes endogenous levels of total Gasdermin D protein. This antibody recognizes the 30 kDa amino terminal fragment produced during pyroptosis by caspase-1. Cleaved Gasdermin D (Asp276) (E3E3P) Rabbit mAb recognizes the amino terminal fragment of mouse Gasdermin D protein only when cleaved at Asp276. Caspase-1 (E2Z1C) Rabbit mAb detects endogenous levels of pro-caspase-1 and the p10 subunit of activated caspase-1. Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb detects endogenous levels of caspase-1 only when cleaved at Asp296. A non-specific band is detected at 70 kDa in some cells. IL-1β (D3H1Z) Rabbit mAb (Mouse Specific) recognizes endogenous levels of total mouse IL-1β protein. This antibody can detect 500 pg of mature recombinant mouse IL-1β. Cleaved IL-1β (Asp117) (E7V2A) Rabbit mAb (Mouse Specific) detects endogenous levels of mouse IL-1β protein only when cleaved at Asp117. Caspase-11 (17D9) Rat mAb recognizes endogenous levels of total caspase-11 protein. ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific) recognizes endogenous levels of total mouse ASC/TMS1 protein. HMGB1 (D3E5) Rabbit mAb detects endogenous levels of total HMGB1. It does not cross-react with other HMGB proteins, including HMGB2 and HMGB3.

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.

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

- (1) Frank, D. and Vince, J.E. (2019) *Cell Death Differ* 26, 99-114.
- (2) Shi, J. et al. (2017) *Trends Biochem Sci* 42, 245-54.
- (3) Malik, A. and Kanneganti, T.D. (2017) *J Cell Sci* 130, 3955-63.
- (4) He, W.T. et al. (2015) *Cell Res* 25, 1285-98.
- (5) Shi, J. et al. (2015) *Nature* 526, 660-5.
- (6) Kayagaki, N. et al. (2015) *Nature* 526, 666-71.
- (7) Viganò, E. et al. (2015) *Nat Commun* 6, 8761.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Leu60 of mouse Gasdermin D, His124 of mouse IL-1β, Ala137 of human HMGB1, a peptide near the carboxy terminus of mouse caspase-1, a recombinant fragment specific to the p30 subunit of mouse caspase-11, and a recombinant fragment specific to mouse ASC/TMS1 protein. Cleavage specific monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Asp296 of mouse caspase-1, Asp117 of mouse IL-1β, and Asp276 of mouse Gasdermin D protein.

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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.**