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#43811

# Pyroptosis Antibody Sampler Kit



**Support:** +1-978-867-2388 (U.S.)  
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**For Research Use Only. Not For Use In Diagnostic Procedures.**

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Gasdermin D (E8G3F) Rabbit mAb	97558	20 µl	53, 43, 30, 21 kDa	Rabbit IgG
Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb	36425	20 µl	30 kDa	Rabbit IgG
Caspase-1 (D7F10) Rabbit mAb	3866	20 µl	48, 20 kDa	Rabbit IgG
Cleaved Caspase-1 (Asp297) (D57A2) Rabbit mAb	4199	20 µl	20, 22 kDa	Rabbit IgG
IL-1β (D3U3E) Rabbit mAb	12703	20 µl	17, 31 kDa	Rabbit IgG
Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb	83186	20 µl	17 kDa	Rabbit IgG
Caspase-4 Antibody	4450	20 µl	45 kDa	Rabbit
Caspase-5 (D3G4W) Rabbit mAb	46680	20 µl	50, 44, 35 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](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The 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 (E8G3F) 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, a 43 kDa fragment produced by caspase-3, as well as a 21 kDa fragment produced by cleavage at both sites. Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb detects the N-terminal fragment of human Gasdermin D protein only when cleaved at Asp275. Caspase-1 (D7F10) Rabbit mAb detects endogenous levels of full length human caspase-1 and the activated p20 subunit by overexpression. Cleaved Caspase-1 (Asp297) (D57A2) Rabbit mAb detects the p20 subunit of human caspase-1 upon cleavage at Asp275. IL-1β (D3U3E) Rabbit mAb can recognize endogenous levels of full-length protein, but does not detect endogenous levels of the mature IL-1β. It can detect up to 100 pg of recombinant mature IL-1β. Cleaved IL-1β (Asp116) (D3A3Z) Rabbit mAb detects endogenous levels of mature IL-1β protein when cleaved at Asp116. Caspase-4 Antibody detects endogenous levels of total caspase-4 and intermediate forms at 40 and 32 kDa. Caspase-5 (D3G4W) Rabbit mAb detects endogenous levels of caspase-5. 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 antibody.

Please visit [www.cellsignal.com](http://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 Trp130 of human Gasdermin D, residues within the p20 subunit of caspase-1, Asp297 of human caspase-1, Asp116 of human IL-1β, Pro154 of human caspase-5, and Ala137 of human HMGB1. IL-1β (D3U3E) Rabbit mAb is produced by immunizing animals with a recombinant IL-1β protein. Polyclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ile125 of human caspase-4. Polyclonal Antibodies are purified by protein A and peptide affinity chromatography.

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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.**