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insights

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Gasdermin D Signaling & Inflammasome Research

Pyroptosis is an inflammatory programmed cell death that is initiated in response to pathogen- or host-derived perturbations of the cytosol. In human and mice pyroptosis is induced by inflammatory caspases, such as caspase-1 and -11 that are activated by inflammasomes. Gasdermin D (GSDMD) is a central mediator of pyroptotic cell death. It contains a functional N-terminal domain and an inhibitory C-terminal domain. Upon caspase-1/11 cleavage of the linker located between the two domains of Gasdermin D, the cleaved N-terminal fragment of Gasdermin D oligomerizes and forms pores on the host cell membrane, leading to cell death called pyroptosis.

AdipoGen Life Sciences is the leading manufacturer and supplier of inflammasome signaling research reagents. The validated high quality inflammasome research tools are used and published daily by the experts in the inflammasome research field.

A New Tool to Measure Pyroptosis:

UNIQUE

Gasdermin D (mouse) ELISA Kit

The Gasdermin D (mouse) ELISA Kit (Prod. No. AG-45B-0011) is a sandwich ELISA for quantitative determination of mouse Gasdermin D in cell culture supernatants and in cell extracts. This ELISA is specific for the measurement of natural and recombinant mouse Gasdermin D (full-length and C-terminus cleaved fragment). It does not detect human Gasdermin D.

NEW **Gasdermin D (mouse) ELISA Kit**

AG-45B-0011 96 wells

Detects full-length and cleaved C-terminal mouse Gasdermin D in cell culture supernatants and cell extracts. Does not cross-react with human Gasdermin D.

Sensitivity: 14 pg/ml
Range: 15.6 to 1000 pg/ml
Sample: Cell Culture Supernatant, Cell Lysate

FIGURE 1

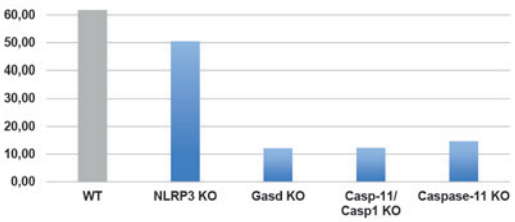
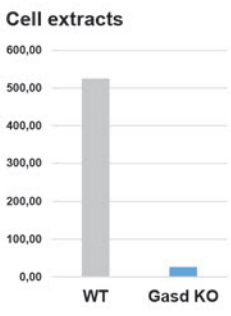


FIGURE 2



Specificity:

Gasdermin D is tested from supernatants of Bone Marrow-Derived Macrophages cells (BMDMs) transfected with LPS from different knockout mice strains (see Figure 1). Only the supernatants from WT and NLRP3$^{-/-}$ strains contain the protein Gasdermin D. Gasdermin D is also tested from cell extracts (lysed with a Triton X-100 buffer) of Bone Marrow-Derived Macrophages cells from WT and Gasdermin D knockout mice strains (see Figure 2).

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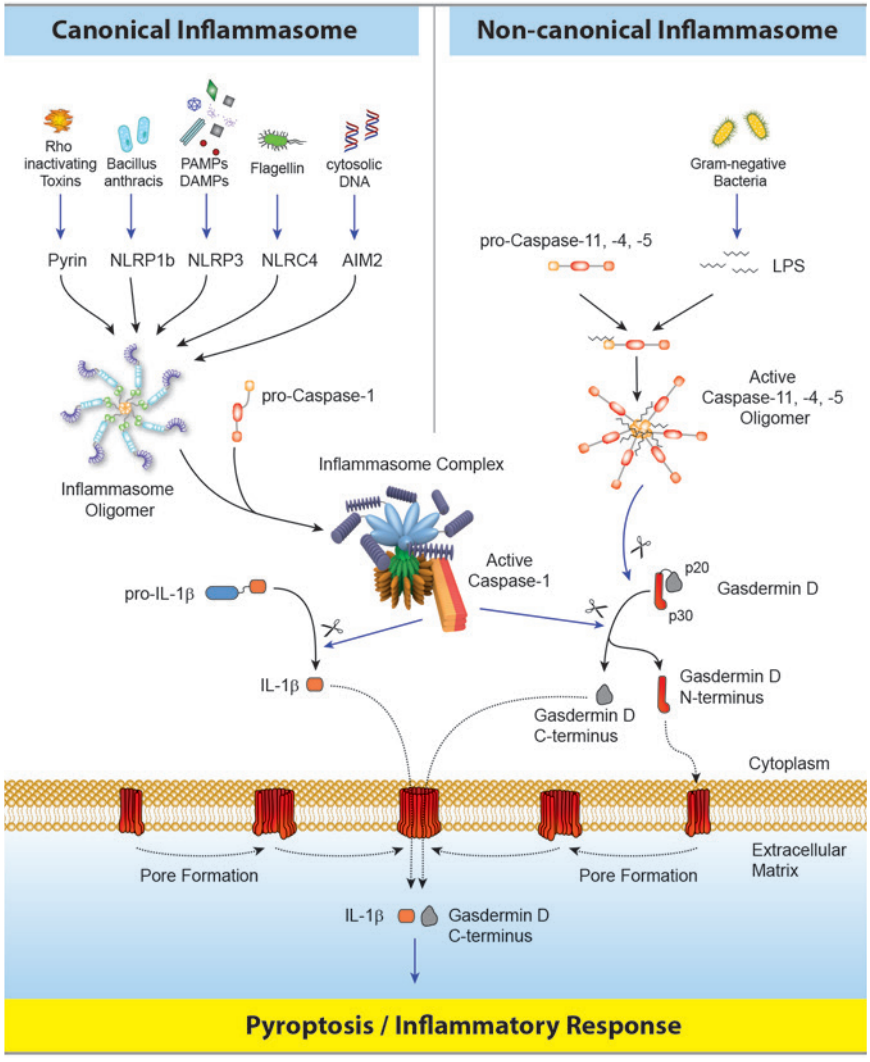
Gasdermin D Signaling Pathways

Inflammasomes are multimeric protein complexes that comprise a sensor (e.g. NLRP3), an adaptor (ASC/Pycard) and a protease (pro-caspase-1) (1). An inflammasome assembles in response to a diverse range of pathogen-associated or danger-associated molecular patterns (PAMPs or DAMPs), or perturbations in cytoplasmic homeostasis (term "homeostasis-altering molecular processes" (HAMPs)) (2). The inflammasome platform leads to activation of caspase-1, which further induces maturation of interleukin-1β and -18 (IL-1β and IL-18) through proteolytic cleavage of pro-IL-1β and pro-IL-18. Activated caspase-1, and also the recently characterized caspase-11 non-canonical inflammasome pathway, cleave the newly discovered intracellular protein Gasdermin D (3, 4). The Gasdermin family members contain N-terminal domains that are capable of forming membrane pores, whereas the C-terminal domains of Gasdermins function as inhibitors of such cytolysis through intramolecular domain association. Caspase-1 or -11 cleavage of Gasdermin D is required for regulation of Pyroptosis: upon caspase-1/11 cleavage of the Gasdermin N- and C-domain linker, the cleaved N-terminal fragment of Gasdermin D oligomerizes and forms pores on the host cell membrane (5), leading to a cell death called pyroptosis and further activation of inflammasomes by triggering K^+ efflux (6). Gasdermin D forming pores regulate the non-conventional secretion of cytokines such as IL-1β, in response to cytosolic LPS and other activators of the inflammasome (7). Neutrophil extrusion of neutrophil extracellular traps (NETs) and concomitant cell death (NETosis), a particular neutrophil defense against pathogens, are dependent on Gasdermin D cleavage by caspase-11 (8). Gasdermin D-mediated pyroptosis is regulated at the level of lipid peroxidation (9) and seems to be a key effector in the LPS-induced lethal sepsis (10).

Caspase-8, an upstream activator of caspase-3, controls apoptotic cell death and prevents RIPK3–MLKL-dependent necroptosis. Caspase-8, activated by the Yersinia effector protein YopJ, also triggers Gasdermin D processing and cell death with different Yersinia species (11).

After formation of the pore at the cellular membrane by Gasdermin D N-terminal fragment, the role and fate of the C-terminus fragment of Gasdermin D is still unclear. Using the Gasdermin D (mouse) ELISA Kit (Prod. No. AG-45B-0011), that detects the C-terminal part of Gasdermin D (as well as the full-length protein), a signal is detected in the supernatant of cells dying by pyroptosis, suggesting that the C-terminal fragment is released from cells, either by chance due to the presence of a pore or for a specific task not yet clear.



LITERATURE REFERENCES: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta: F. Martinon, et al.; Mol. Cell **10,** 417 (2002) • Homeostasis-altering molecular processes as mechanisms of inflammasome activation: A. Liston & S.L. Masters; Nat. Rev. Immunol. **17,** 208 (2017) • Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling: N. Kayagaki, et al.; Nature **526,** 666 (2015) • Mechanisms of Gasdermin Family Members in Inflammasome Signaling and Cell Death: S. Feng, et al.; J. Mol. Biol. **430,** 3068 (2018) • Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores: X. Liu, et al.; Nature **535,** 153 (2016) • Gasdermin D Restrains Type I Interferon Response to Cytosolic DNA by Disrupting Ionic Homeostasis: I. Banerjee, et al.; Immunity **49,** 413 (2018) • The Pore-Forming Protein Gasdermin D Regulates Interleukin-1 Secretion from Living Macrophages: C.L. Evavold, et al.; Immunity **48,** 35 (2018) • Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps: K.W. Chen, et al.; Sci. Immunol. **3,** 26 (2018) • Lipid Peroxidation Drives Gasdermin D-mediated Pyroptosis in Lethal Polymicrobial Sepsis: R. Kang, et al.; Cell Host Microbe **24,** 97 (2018) • Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis: J.K. Rathkey, et al.; Sci. Immunol. **3,** 26 (2018) • Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death: P. Orning, et al.; Science **(Epub ahead of print)** 26 (2018)

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APPLICATIONS: FACS: Flow Cytometry; **FUNC:** Functional Application; **ICC:** Immunocytochemistry; **IHC:** Immunohistochemistry **IP:** Immunoprecipitation; **WB:** Western blot

FORMULATION: PF = Preservative free

SPECIES: Hu = Human; **Ms** = Mouse; **Rt** = Rat; **Rb** = Rabbit; **Prm** = Primate

The Standard Inflammasome Research Antibodies - *Validated Reliable Antibodies used by the Experts*

PRODUCT NAME	PID	PRODUCT DESCRIPTION
anti-Caspase-1 (p20) (mouse), mAb (Casper-1)	AG-20B-0042	Recognizes endogenous full-length and activated (p20 fragment) mouse caspase-1.
anti-Caspase-1 (p10) (mouse), mAb (Casper-2)	AG-20B-0044	Recognizes endogenous full-length and activated (p10 fragment) mouse caspase-1.
anti-Caspase-1 (p20) (human), mAb (Bally-1)	AG-20B-0048	Recognizes endogenous full-length and activated (p20 fragment) human caspase-1.
anti-NLRP3/NALP3, mAb (Cryo-2)	AG-20B-0014	Recognizes mouse and human NLRP3/NALP3. ***Over 1000 Citations!***
anti-Asc, pAb (AL177)	AG-25B-0006	Recognizes human and mouse Asc.
anti-IL-1α (p18) (mouse), mAb (Teo-1)	AG-20B-0064	Recognizes full-length and cleaved p18 fragment of mouse IL-1α.
anti-Caspase-8 (human), mAb (C15)	AG-20B-0057	Recognizes the p18 subunit of human caspase-8.
anti-Caspase-8 (mouse), mAb (1G12)	AG-20T-0137	Recognizes full-length and the p18 cleaved fragment of mouse caspase-8.
anti-Caspase-8 (mouse), mAb (3B10)	AG-20T-0138	Recognizes full-length and the p18 cleaved fragment of mouse caspase-8.

For Inflammasome Research Protocols using AdipoGen Life Sciences' Validated Antibodies visit our Inflammasome "Insights" Page.

Key Inflammasome Activators and Inhibitors

PRODUCT NAME	PID	PRODUCT DESCRIPTION
Monosodium urate (crystals)	AG-CR1-3950	Potent NLRP3 inflammasome activator.
Monosodium urate (ready-to-use)	AG-CR1-3951	Potent NLRP3 inflammasome activator.
Nigericin . Na	AG-CN2-0020	Potent NLRP3 inflammasome activator.
N-Acetyl-D-glucosamine	AG-CN2-0489	Acts as activator of NLRP3 inflammasome by dissociating the enzyme hexokinase from the mitochondria.
MCC950 . Na (water soluble)	AG-CR1-3615	Potent and selective NLRP3 inflammasome inhibitor.
Isoliquiritigenin	AG-CN2-0459	Inhibits NLRP3-activated Asc oligomerization. Blocks priming and activation steps.
BAY 11-7082	AG-CR1-0013	Reduces ATPase activity of the NLRP3 inflammasome.
(R)-3-Hydroxybutyric acid	AG-CR1-3616	Prevents K^+-efflux and consequently reduce Asc oligomerization and speck formation.
(S)-3-Hydroxybutyric acid	AG-CR1-3617	
DL-3-Hydroxybutyric acid sodium salt	CDX-H0080	
NEW K777 [K11777]	AG-CR1-0158	Broad-range cathepsin inhibitor useful for inflammasome inhibition.

Priming Step of Inflammasome Activation – Simple and Convenient!

Visit www.adipogen.com to see a Panel of ready-to-use LPS Solutions for Inflammasome Priming Activation. Do not bother anymore to solubilize your LPS, choose and use AdipoGen Life Sciences' homogenous ready-to-use LPS solutions.

NEW

Unique Gasdermin D (mouse-specific) Antibody

Detects cleaved C-terminal and full-length Gasdermin D

AdipoGen Life Sciences' anti-Gasdermin D (mouse), pAb (IN110) (Prod. No. AG-25B-0036) is a polyclonal antibody immunized with the recombinant C-terminus domain of mouse Gasdermin D. **The antibody recognizes full-length and the cleaved C-terminus of mouse Gasdermin D,** does not cross-react with human Gasdermin D and works specifically in Western Blot application to detect the cleaved C-terminal Gasdermin D.

NEW **anti-Gasdermin D (mouse), pAb (IN110)**

AG-25B-0036 100 µg

Source	Guinea pig
Immunogen	Recombinant mouse gasdermin D (C-terminus).
Application	ELISA, WB
Specificity	Recognizes full-length and cleaved C-terminus domain of mouse gasdermin D. Does not cross-react with human gasdermin D.

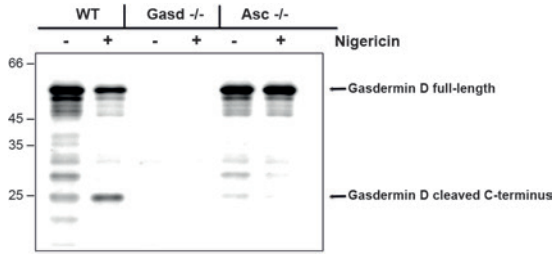


FIGURE: Mouse Gasdermin D (full-length and cleaved p22 fragments) are detected by immunoblotting using anti-Gasdermin D (mouse), pAb (IN110) (Prod. No. AG-25B-0036).

METHOD: Gasdermin D is analyzed by Western blot in cell extracts of bone marrow-derived macrophage cells (BMDMs) (WT, Gasdermin -/- or Asc -/-) treated with LPS (50ng/ml; Prod. No. AG-CU1-0001) for 3h and +/- Nigericin (5µM for 2.5h, Prod. No. AG-CN2-0020). Cell extracts are separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Gasdermin D (mouse), pAb (IN110) (0.5µg/ml). After addition of an anti-guinea pig secondary antibody coupled to HRP (1/5000), proteins are visualized by a chemiluminescence detection system.

Picture courtesy of Prof. Olaf Gross, University Medical Center Freiburg, Germany

NEW

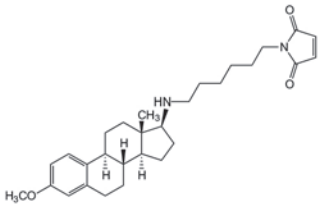
Gasdermin D-induced Pyroptosis Inhibitors

U-73122 is a potent cell permeable phospholipase C (PLC) and SERCA inhibitor that has recently been shown to be a useful inflammasome research reagent by acting as a Gasdermin D N-terminal fragment (GSDMD-N)-induced pyroptosis inhibitor. It protects against GSDMD-N cytotoxicity in macrophages or against lethal infection in mice.

NEW **U-73122**

AG-CR1-3698 1 mg | 5 mg

Formula	$C_{29}H_{40}N_2O_3$
MW	464.6
CAS	112648-68-7



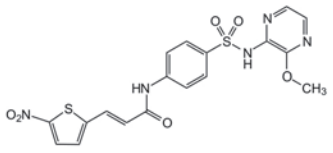
LIT: Lipid peroxidation drives Gasdermin D-mediated pyroptosis in lethal polymicrobial Sepsis: R. Kang, et al.; Cell Host Microbe **24,** 97 (2018)

Necrosulfonamide is a potent inhibitor of necroptosis, by blocking the mixed lineage kinase domain-like protein (MLKL) has recently been shown to act as an inhibitor of Gasdermin D that works well for mice studies. It binds directly to Gasdermin D and inhibits the oligomerization of the N-terminus and therefore the pore formation and pyroptosis.

NEW **Necrosulfonamide**

AG-3705 5 mg | 25 mg

Formula	$C_{18}H_{15}N_5O_6S_2$
MW	461.5
CAS	1360614-48-7



LIT: Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis: J.K. Rathkey, et al.; Sci. Immunol. **3,** eaat2738 (2018)

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