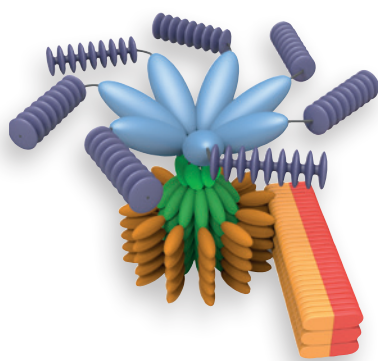


THE EXPERT IN

2<sup>nd</sup> Edition

# Inflammasome Research

From Innate to Adaptive Immunity



**Inflammasomes** are multi-protein complexes whose activity has been implicated in physiological and pathological inflammation. The hallmarks of inflammasome activation are the secretion of the mature forms of caspase-1 and interleukin-1 $\beta$  (IL-1 $\beta$ ) from cells of the innate immune system. An inflammasome represents a high molecular weight complex that activates inflammatory caspases and cytokines of the IL-1 family (IL-1 $\beta$ , IL-18 and depending on the stimulus also IL-1 $\alpha$ ). Several inflammasomes have been described which contain different sensor proteins such as **NLRP1** (NALP1), **NLRP3** (NALP3), **IPAF** (NLRC4), **NLRP6** (NALP6), **NLRP12** (NALP12), **RIG-I** and **AIM-2** (absent in melanoma 2). Most of these inflammasomes require the adapter protein **Asc** (apoptosis-associated speck-like protein containing a caspase recruitment domain) to recruit caspase-1 to the inflammasome complex. Upon binding to the inflammasome caspase-1 is cleaved and activated, leading to cleavage of its various targets and causing maturation and secretion of the pro-inflammatory IL-1 $\beta$ . Inflammasomes can be activated through multiple signals including live bacteria, microbial toxins, xeno-compounds, particulates cytoplasmic pathogen-associated

molecular patterns (**PAMPs**) and/or endogenous danger signals (**DAMPs**).

Inflammasome activity has been causally linked to the induction of numerous inflammatory responses, which can be either beneficial or harmful to the organism. Beneficial responses arise by maintaining homeostatic tissue function (detection and repair of tissue damages after trauma or pathogen invasion). Among the harmful inflammatory responses are particle-induced sterile inflammation, caused by host-derived particles such as monosodium urate (MSU) crystals, which are involved in the pathogenesis of gout, as well as environmental and industrial particles such as asbestos, silica and metallic nanoparticles, which induce lung inflammation upon inhalation. Accumulating evidence also implicates inflammasome activity in numerous other diseases, including cancer and the development of metabolic diseases (like type 2 diabetes, atherosclerosis), some neurodegenerative diseases (like Alzheimer, Prion, Parkinson), autoimmune diseases (such as multiple sclerosis) and inflammatory bowel diseases. Beneficial effects for the host include the enhancement of vaccine efficacy.

## SELECTED REVIEW ARTICLES

Molecular mechanisms of inflammasome signaling: A. Mathur, et al.; *J. Leukoc. Biol.* **103**, 233 (2018) • Inflammasome activation and assembly at a glance: A. Malik & T.D. Kanneganti; *J. Cell Sci.* **130**, 3955 (2017) • Inflammasomes: mechanism of action, role in disease, and therapeutics: H. Guo, et al.; *Nat. Med.* **21**, 677 (2015) • Activation and regulation of the inflammasomes: E. Latz, et al.; *Nat. Rev. Immunol.* **13**, 397 (2013) • The inflammasome: an integrated view: O. Gross, et al.; *Immunol. Rev.* **243**, 136 (2011)

## THE STANDARD

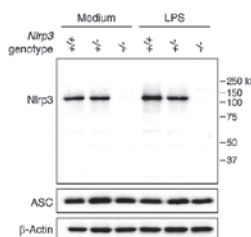
### NLRP3 Antibody

#### anti-NLRP3/NALP3, mAb (Cryo-2)

AG-20B-0014-C100

100  $\mu$ g

<b>Clone</b>	Cryo-2
<b>Isotype</b>	Mouse IgG2b
<b>Immunogen</b>	Recombinant mouse NLRP3/NALP3 (pyrin domain/aa 1-93).
<b>Application</b>	ICC, IHC, IP, WB (1 $\mu$ g/ml) (see online protocol)
<b>Specificity</b>	Recognizes human and mouse NLRP3/NALP3.



**FIGURE:** Mouse NLRP3 is detected in mouse macrophages using the monoclonal antibody to NLRP3 (Cryo-2) (Prod. No. AG-20B-0014).

## Inflammasome Tools

<b>Caspase-1 Detection</b>	<b>2, 5</b>
<b>Standard Antibodies</b>	<b>3</b>
<b>Signaling Chart</b>	<b>4</b>
<b>Priming</b>	<b>5</b>
<b>Microtubule Assembly</b>	<b>6</b>
<b>Inhibitors/Activators</b>	<b>7-8</b>
<b>Flagellin</b>	<b>8</b>

# Specific Caspase-1 Detection

MONOCLONAL ANTIBODIES FROM THE EXPERTS & VALIDATED BY KEY LABORATORIES!

## Unique Antibodies to Detect Activated (p10&p20) Mouse Caspase-1 by WB

- Purified mouse monoclonal antibodies (mAbs)
- Casper-1 detects the endogenous full-length & activated p20 fragment
- Casper-2 detects the endogenous full-length & activated p10 fragment
- Outstanding tools to monitor inflammasome activation
- Tested by experts in the inflammasome signaling field
- No protein precipitation from supernatants is required

### THE STANDARD anti-Caspase-1 (p10) (mouse), mAb (Casper-2)

AG-20B-0044-C100 100 µg  
AG-20B-0044B-C100 Biotin 100 µg

**Clone** Casper-2  
**Isotype** Mouse IgG2a  
**Immunogen** Recombinant mouse caspase-1  
**Application** WB (1 µg/ml) (see online protocol)  
**Specificity** Recognizes endogenous full-length and activated (p10 fragment) mouse caspase-1.

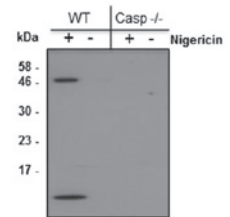
### THE STANDARD anti-Caspase-1 (p20) (mouse), mAb (Casper-1)

AG-20B-0042-C100 100 µg  
AG-20B-0042B-C100 Biotin 100 µg

**Clone** Casper-1  
**Isotype** Mouse IgG1  
**Immunogen** Recombinant mouse caspase-1  
**Application** WB (1 µg/ml) (see online protocol), IHC (PS), IP  
**Specificity** Recognizes endogenous full-length and activated (p20 fragment) mouse caspase-1.

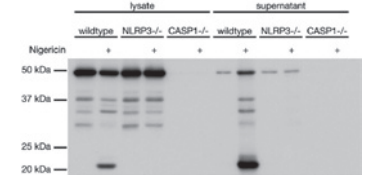
**PROTOCOLS FOR CASPER-1, CASPER-2, BALLY-1 AND CRYO-2:** Measuring the inflammasome: O. Gross; *Methods Mol. Biol.* **844**, 199 (2012) • Immunoblotting for active caspase-1: C. Jakobs, et al.; *Methods Mol. Biol.* **1040**, 103 (2013) • Measuring NLR Oligomerization I: Size Exclusion Chromatography, Co-immunoprecipitation, and Cross-Linking: S. Khare, et al.; *Methods Mol. Biol.* **1417**, 131 (2016) • Assessing Caspase-1 Activation: B. Guey & V. Pettrilli; *Methods Mol. Biol.* **1417**, 197 (2016) • Cell-Free Assay for Inflammasome Activation: Y. Jamilloux & F. Martinon; *Methods Mol. Biol.* **1417**, 207 (2016)

**FIGURE:** Mouse caspase-1 (p10) is detected by immunoblotting using anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (Prod. No AG-20B-0044).



**METHOD:** Caspase-1 was analyzed by Western blot in supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type and caspase-1<sup>-/-</sup> mice activated or not by 5 µM nigericin (Prod. No. AG-CN2-0020) for 30 min. Supernatants (30 µl) were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (1 µg/ml). Proteins were visualized by a chemiluminescence detection system.

**FIGURE:** Mouse caspase-1 (p20) is detected by immunoblotting using anti-Caspase-1 (p20) (mouse), mAb (Casper-1) (Prod. No. AG-20B-0042).



**METHOD:** Caspase-1 was analyzed by Western blot in cell extracts and supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type, NLRP3<sup>-/-</sup> and caspase-1<sup>-/-</sup> mice activated or not by 5 µM nigericin (Prod. No. AG-CN2-0020) for 30 min. Cell extracts and supernatants were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p20) (mouse), mAb (Casper-1) (1 µg/ml). Proteins were visualized by a chemiluminescence detection system.

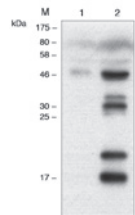
## Antibody to Detect Activated (p20) Human Caspase-1 by WB

### THE STANDARD anti-Caspase-1 (p20) (human), mAb (Bally-1)

AG-20B-0048-C100 100 µg  
AG-20B-0048B-C100 Biotin 100 µg

**Clone** Bally-1  
**Isotype** Mouse IgG1  
**Immunogen** Recombinant human caspase-1  
**Application** WB (1 µg/ml) (see online protocol)  
**Specificity** Recognizes endogenous full-length and activated (p20 fragment) human caspase-1.

**FIGURE:** Human caspase-1 (p20) is detected by immunoblotting using anti-Caspase-1 (p20) (human), mAb (Bally-1) (Prod. No AG-20B-0048).



**METHOD:** Caspase-1 was analyzed by Western blot in supernatants of THP1 cells differentiated for 3h with 0.5 µM PMA (Prod. No. AG-CN2-0010) and activated (lane 2) or not (lane 1) by 5 µM Nigericin for 1h (Prod. No. AG-CN2-0020). Supernatants (30 µl) were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p20) (human), mAb (Bally-1) (1 µg/ml). Proteins were visualized by a chemiluminescence detection system.

## Key Caspase-1 Inhibitors

### Z-VAD-FMK (Cell permeable)

AG-CP3-0002-M001 1 mg  
AG-CP3-0002-M005 5 mg

**LI:** Malarial hemozoin is a Nalp3 inflammasome activating danger signal; C. Dostert, et al.; *PLoS One* **4**, e6510 (2009)

### Q-VD-Oph

AG-CP3-0006-M001 1 mg  
AG-CP3-0006-3001 3 x 1 mg  
AG-CP3-0006-M005 5 mg

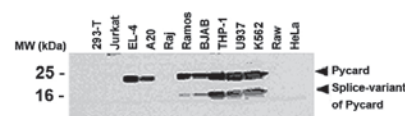
*For Negative Control see Q-VE-Oph (Prod. No. AG-CP3-0007).*

## Asc Antibody AL177

### anti-Asc, pAb (AL177)

AG-25B-0006-C100		100 µg
AG-25B-0006PF-C100	Preservative free	100 µg
AG-25B-0006TS-C100	ATTO 647N	100 µg

<b>Source</b>	Rabbit
<b>Immunogen</b>	Synthetic peptide corresponding to aa at the N-terminal human Asc.
<b>Application</b>	ICC, IHC (PS), IP, WB, FUNC (Inhibition)*
<b>Specificity</b>	Recognizes human and mouse Asc. * Inhibits interaction between Asc and NLRP3, leading to blockade of caspase-1 processing <i>in vitro</i> .



**FIGURE:** Western blot analysis of human and mouse cell lines using anti-Asc, pAb (AL177) (Prod. No. AG-25B-0006). Total protein extracts from various human (293-T, Jurkat, Raj, Ramos, BJAB, THP-1, U937, K562, Raw, HeLa) and mouse (EL-4, A20) cell lines were run on SDS-PAGE and Pycard detected by anti-Asc, pAb (AL177) at 1:1'000 dilution. Anti-rabbit IgG coupled horse radish peroxidase was used at 1:5'000 dilution for ECL detection.

**PROTOCOLS FOR AL177:** Measuring inflammasome activation in response to bacterial infection: P. Broz & D.M. Monack; *Methods Mol. Biol.* **1040**, 65 (2013) • Measuring NLR Oligomerization II: Detection of ASC Speck Formation by Confocal Microscopy and Immunofluorescence: M. Beilharz, et al.; *Methods Mol. Biol.* **1417**, 145 (2016) • Cell-Free Assay for Inflammasome Activation: Y. Jamilloux & F. Martinon; *Methods Mol. Biol.* **1417**, 207 (2016)

### NEW Asc Antibody (AL177) Blocking Peptide

AG-37B-0001-C100	100 µg
------------------	--------

Blocking Peptide for anti-Asc, pAb (AL177). This vial contains 100µg peptide in 100µl sterile water. The Asc Antibody (AL177) Blocking Peptide can be used in conjunction with anti-Asc pAb (AL177) (Prod. No. AG-25B-0006) to block protein-antibody complex formation.

### NEW Asc (AL177) Antibody + Blocking Peptide Set

AG-44B-2000-KI01	1 Set
------------------	-------

The Asc (AL177) Antibody + Blocking Peptide Set contains one vial each of the anti-Asc, pAb (AL177) (Prod. No. AG-25B-0006) and the Asc Antibody (AL177) Blocking Peptide (Prod. No. AG-37B-0001).

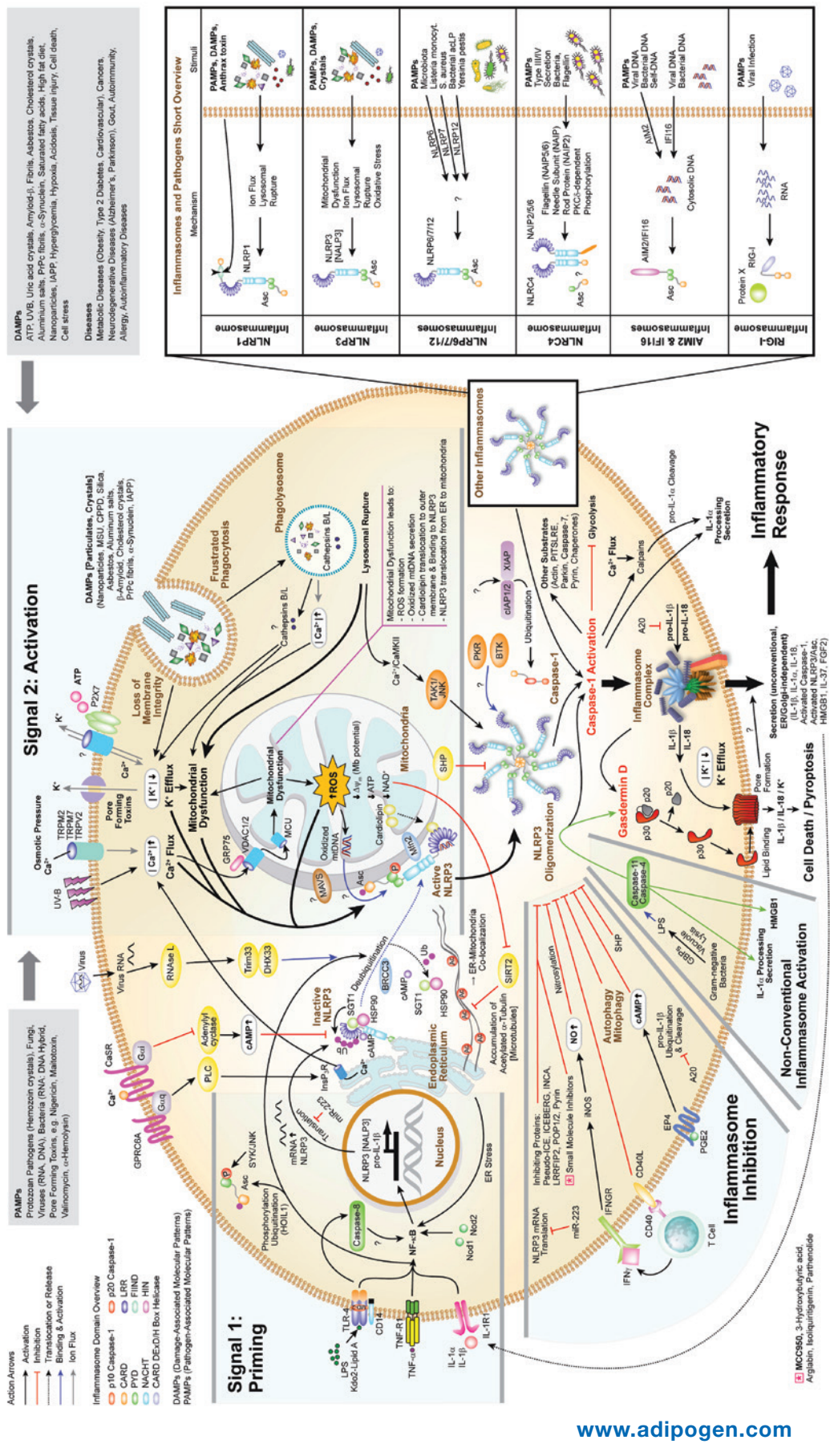
## Standard Inflammasomes Signaling Antibodies

PRODUCT NAME	PID	SIZE	SOURCE/ISOTYPE	SPECIES	APPLICATION
<b>Nod-like Receptors (NLRs)</b>					
<b>anti-NAIP1/2/5 (mouse), mAb (Naipa-1)</b>	AG-20B-0045	100 µg	Mouse IgG2bκ	Ms	WB
<b>anti-NLRP1/NALP1 (human), pAb (AL176)</b>	AG-25B-0005	100 µg	Rabbit	Hu	WB
<b>anti-NLRP3/NALP3, mAb (Cryo-2)</b>	AG-20B-0014	100 µg	Mouse IgG2b	Hu, Ms	ICC, IHC, IP, WB
<b>anti-NLRP3/NALP3 (mouse), mAb (Cryo-1)</b>	AG-20B-0006	100 µg	Mouse IgG2b	Ms	WB
<b>anti-NLRP6/NALP6 (human), mAb (Clint-1)</b>	AG-20B-0046	100 µg	Mouse IgG1κ	Hu	WB
<b>RIG-like Helicases (RLHs) – Antiviral Signaling</b>					
<b>anti-RIG-I, mAb (Alme-1)</b>	AG-20B-0009	100 µg	Mouse IgG1	Hu, Ms	IHC, IP, WB
<b>anti-RIG-I, mAb (Alme-1) (Biotin)</b>	AG-20B-0009B	100 µg	Mouse IgG1	Hu, Ms	IHC, IP, WB
<b>anti-Cardif (human), mAb (Adri-1)</b>	AG-20B-0004	100 µg	Mouse IgG2b	Hu	ICC, IHC, IP, WB
<b>anti-MDA5 (human), mAb (Hely-1)</b>	AG-20B-0013	100 µg	Mouse IgG1	Hu	ELISA, IP, WB
<b>anti-NS3 (HCV), mAb (1B6)</b>	AG-20B-0001	100 µg	Mouse IgG1	HCV	ICC, WB
<b>anti-NS5B (HCV), mAb (5B-3B1)</b>	AG-20B-0002	100 µg	Mouse IgG2b	HCV	WB
<b>anti-NS5B (HCV), mAb (blocking) (5B-12B7)</b>	AG-20B-0003	100 µg	Mouse IgG2a	HCV	ICC, IP, FUNC (Blocking)
<b>Cytosolic DNA Sensor</b>					
<b>anti-AIM2 (human), mAb (3B10)</b>	AG-20B-0040	100 µg	Mouse IgG1	Hu	ICC, WB
<b>Signaling Antibodies</b>					
<b>anti-Pyrin (human), pAb (AL196)</b>	AG-25B-0020	100 µg	Rabbit	Hu	IP, WB
<b>Cytosolic PAMPs Sensors</b>					
<b>anti-Caspase-4/11 (p20), mAb (Flamy-1)</b>	AG-20B-0060	100 µg	Mouse IgG2bκ	Hu, Ms	IP, WB
<b>anti-Caspase-4/11 (p20), mAb (Flamy-1) (Biotin)</b>	AG-20B-0060B	100 µg	Mouse IgG2bκ	Hu, Ms	IP, WB

# NLRP3 Inflammasome Activation, Signaling & Regulation

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www.adipogen.com

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LIFE SCIENCES



www.adipogen.com

## Caspase-1 – Quantitative Measurement of Inflammasome Activation

A quantitative detection method, alternative to Western blotting, to measure inflammasome activation leading to caspase-1 cleavage and secretion. How to measure inflammasome activation? See our manual on [www.adipogen.com](http://www.adipogen.com).

### Caspase-1 (mouse) Matched Pair Detection Set

AG-46B-0003-KI01 For 5 x 96 well plates

<b>Specificity</b>	Detects mouse caspase-1 (p10 and p20 domain).
<b>Species Reactivity</b>	Mouse
<b>Sensitivity</b>	100 pg/ml
<b>Range</b>	0.15 ng/ml to 10 ng/ml
<b>Assay Type</b>	Colorimetric/Sandwich
<b>Sample Type</b>	Cell Culture Supernatant

### Caspase-1 (mouse) ELISA Kit

AG-45B-0002-KI01 96 wells

<b>Specificity</b>	Detects mouse caspase-1 (p10 and p20 domain).
<b>Species Reactivity</b>	Mouse
<b>Sensitivity</b>	33 pg/ml
<b>Range</b>	15 to 1000 pg/ml
<b>Assay Type</b>	Colorimetric/Sandwich
<b>Sample Type</b>	Cell Culture Supernatant, Serum, Plasma

## Best Antibody to Detect Cleaved mouse IL-1 $\alpha$ (p18) by WB

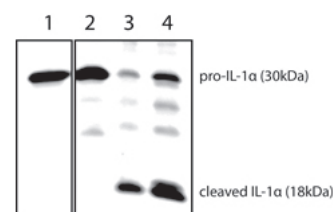
### **NEW** anti-IL-1 $\alpha$ (p18) (mouse), mAb (Teo-1)

AG-20B-0064-C100 100  $\mu$ g

<b>Isotype</b>	Mouse IgG
<b>Application</b>	WB (1 $\mu$ g/ml)
<b>Specificity</b>	Recognizes mouse IL-1 $\alpha$ p18 cleaved and full-length fragments.

**FIGURE:** Mouse IL-1 $\alpha$  (full-length p30 and cleaved p18 fragments) are detected by immunoblotting using anti-IL-1 $\alpha$  (p18) (mouse), mAb (Teo-1) (Prod. No AG-20B-0064).

**METHOD:** IL-1 $\alpha$  was analyzed by Western blot in cell extracts of bone marrow-derived dendritic cells (BMDCs) treated with LPS and several inflammasome activators as indicated. Cell extracts were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-IL-1 $\alpha$  (p18) (mouse), mAb (Teo-1) (1  $\mu$ g/ml). After addition of an anti-mouse secondary antibody coupled to HRP, proteins were visualized by a chemiluminescence detection system.



- 1: Lysate of LPS-primed BMDCs
- 2: Supernatant of LPS + ATP treated BMDCs
- 3: Supernatant of LPS + Nigericin treated BMDCs
- 4: Supernatant of LPS + MSU treated BMDCs

For more Inflammasome Signaling & IL-1-related Products visit [www.adipogen.com](http://www.adipogen.com)

## Inflammasome “Priming” Activators

### Priming of the NLRP3 Inflammasome

The most prominent function of the **NLRP3 inflammasome** is the processing and activation of pro-interleukin-1 $\beta$  (pro-IL-1 $\beta$ ). Yet most cells do not express pro-IL-1 $\beta$  and thus prior expression of pro-IL-1 $\beta$  is required. This can be achieved by stimulating receptors such as TLRs (e.g. through LPS), NODs, TNF-Rs (e.g. through TNF- $\alpha$ ) or IL-1R1 (through IL-1 $\alpha$  and IL-1 $\beta$ ) that activate NF- $\kappa$ B and initiate pro-IL-1 $\beta$  transcription. This process of pro-IL-1 $\beta$  induction is called **priming (Signal 1)**. Priming also induces NF- $\kappa$ B-dependent transcription of NLRP3.

An additional stimulus (**Signal 2**) results in the activation of the NLRP3 inflammasome and subsequent initiation of downstream signaling. In the absence of priming, NLRP3 inflammasome-dependent caspase-1 activation can also be observed, but IL-1 $\beta$  secretion is absent.

**FOR DETAILS SEE:** Inflammasome Priming in Sterile Inflammatory Disease: M.N. Patel, et al.; Trends Mol. Med. **23**, 165 (2017) • Critical functions of priming and lysosomal damage for NLRP3 activation: V. Hornung & E. Latz; Eur. J. Immunol. **40**, 620 (2010) • The inflammasomes: K. Schröder & J. Tschopp; Cell **140**, 821 (2010)

### TNF- $\alpha$ , Soluble (human) (rec.)

AG-40B-0006 10  $\mu$ g | 50  $\mu$ g | 3 x 50  $\mu$ g

### TNF- $\alpha$ (human) (multimeric) (rec.)

AG-40B-0019 10  $\mu$ g | 3 x 10  $\mu$ g

### TNF- $\alpha$ (mouse) (multimeric) (rec.)

AG-40B-0021 10  $\mu$ g | 3 x 10  $\mu$ g

### Lipopolysaccharides (LPS)

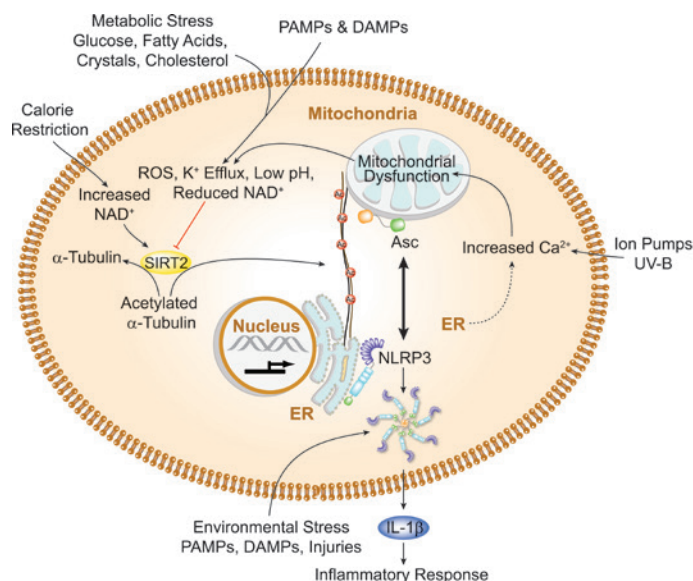
For a full panel see our **Innate Immunity Brochure**



# Microtubules & Inflammasome Complex Assembly

Inflammasomes are assembled from a pattern-recognition receptor, the adapter protein Asc and caspase-1 to process interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 in response to microbial components or damage-associated signals. Recently, it was shown that **microtubules** might have a central role in the assembly of the NOD, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome. Inhibitors of microtubule polymerization (such as colchicine and nocodazole) significantly decrease the levels of IL-1 $\beta$  that is produced in response to NLRP3 inflammasome activators. However, microtubules did not contribute to the activation of the NLRP3 inflammasome in a phagocytosis-dependent manner. Instead, they are required in a dynein-dependent manner for the relocalization of the mitochondria close to the endoplasmic reticulum following stimulation by inducers of the NLRP3 inflammasome. As a result of this microtubule-dependent process, Asc molecules on the mitochondria came into close proximity and could interact with NLRP3 on the endoplasmic reticulum (ER). NLRP3 activators induce microtubule polymerization and acetylation, with concomitant binding of dynein to acetylated  $\alpha$ -tubulin.

As a proposed mechanism, NLRP3 activation leads to mitochondrial dysfunction followed by a decrease of the mitochondrial coenzyme NAD<sup>+</sup> concentration, which in turn inactivates the NAD<sup>+</sup>-dependent  $\alpha$ -tubulin deacetylase sirtuin 2. This results in the accumulation of acetylated  $\alpha$ -tubulin and the subsequent organelle translocation process.



**FIGURE:** Accumulation of acetylated  $\alpha$ -tubulin facilitates the assembly and activation of the inflammasome by opposing Asc on mitochondria to NLRP3 on the endoplasmic reticulum (ER).

**LIT:** Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome: T. Misawa, et al.; Nat. Immunol. 14, 454 (2013)

**UNIQUE**

## Microtubule Antibodies

PRODUCT NAME	PID	SIZE	SOURCE	APPLICATION
<b>anti-<math>\alpha</math>-Tubulin (acetylated), mAb (TEU318)</b>	AG-20B-0068	100 $\mu$ g	Mouse IgG1	ICC, WB
<b>NEW anti-Tubulin (glycylated), pAb (Gly-pep1)</b>	AG-25B-0034	100 $\mu$ g	Rabbit	ICC, IP, WB
<b>anti-Tubulin-GTP, mAb (rec.) (MB11)</b>	AG-27B-0009	100 $\mu$ g	Human IgG2 $\lambda$	ICC
<b>anti-Polyglutamylation Modification, mAb (GT335)</b>	AG-20B-0020	100 $\mu$ g	Mouse IgG1k	EM, ICC, IP, WB
<b>anti-Polyglutamylation Modification, mAb (GT335) (Biotin)</b>	AG-20B-0020B	100 $\mu$ g	Mouse IgG1k	ICC, IP, WB
<b>anti-Polyglutamate chain (polyE), pAb (IN105)</b>	AG-25B-0030	50 $\mu$ g	Rabbit	ICC, WB

## Small Molecule Cytoskeletal Modulators

**THE SOURCE**

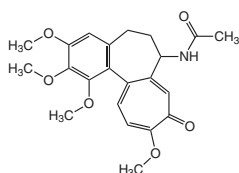
### Colchicine

AG-CN2-0048

500 mg | 1 g

Microtubule inhibitor. Inhibits acetylated  $\alpha$ -tubulin mediated transport of mitochondria and subsequent apposition of Asc on mitochondria to NLRP3 on the endoplasmic reticulum.

**LIT:** Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome: T. Misawa, et al.; Nat. Immunol. 14, 454 (2013)



<b>Dynasore</b>	Dynamain Inhibitor	AG-CR1-0045
<b>Jaspalakinolide</b>	F-actin Stabilization	AG-CN2-0037
<b>Latrunculin A</b>	F-actin Depolymerization	AG-CN2-0027
<b>Latrunculin B</b>	F-actin Depolymerization	AG-CN2-0031
<b>Swinholid A</b>	F-actin Inhibitor	AG-CN2-0035
<b>Cytochalasin B</b>	Actin Depolymerization	AG-CN2-0504
<b>Cucurbitacin E</b>	Actin Depolymerization	AG-CN2-0474
<b>Colcemid</b>	Microtubule Inhibitor	AG-CR1-3567
<b>Ilimaquinone</b>	Microtubule Inhibitor	AG-CN2-0038
<b>Nocodazole</b>	Microtubule Inhibitor	AG-CR1-0019
<b>Paclitaxel</b>	Microtubule Stabilizer	AG-CN2-0045
<b>Phomopsis A</b>	Microtubule Inhibitor	AG-CN2-0515
<b>Podophyllotoxin</b>	Microtubule Inhibitor	AG-CN2-0049
<b>Pseudolaric acid B</b>	Microtubule Inhibitor	AG-CN2-0083

## Key NLRP3 Inflammasome Activators

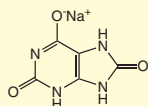
### Monosodium urate

AG-CR1-3950 (crystals) 2 mg | 2x 2 mg  
AG-CR1-3951 (ready-to-use solution) 10 mg

#### Biological Activity Tested!

Potent NLRP3 inflammasome activator.

**LIT:** Gout-associated uric acid crystals activate the NALP3 inflammasome: F. Martinon, et al.; Nature **440**, 237 (2006)



### Nigericin . Na

AG-CN2-0020 5 mg | 25 mg  
Potent NLRP3 inflammasome activator.

**LIT:** Cryopyrin activates the inflammasome in response to toxins and ATP: S. Mariathasan, et al.; Nature **440**, 228 (2006)

### N-Acetyl-D-glucosamine

AG-CN2-0489 250 mg | 1 g | 5 g  
Acts as a new activator of NLRP3 inflammasome by dissociating the enzyme hexokinase from the mitochondria.

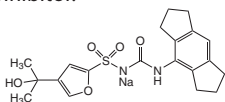
**LIT:** Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan: A.J. Wolf, et al.; Cell **166**, 624 (2016)

## NLRP3 Inflammasome Inhibitors

### MCC950 . Na

AG-CR1-3615 1 mg | 5 mg | 10 mg  
Potent and selective NLRP3 inflammasome inhibitor.

**LIT:** A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases: R.C. Coll, et al.; Nat. Med. **21**, 248 (2015)



### Isoliquiritigenin

AG-CN2-0459 10 mg | 50 mg  
Inhibits NLRP3-activated Asc oligomerization. Blocks priming and activation step.

**LIT:** Isoliquiritigenin is a potent inhibitor of NLRP3 inflammasome activation and diet-induced adipose tissue inflammation: H. Honda, et al.; J. Leukoc. Biol. **96**, 1087 (2014)

### Glyburide (USP)

AG-CR1-3613 1 g | 5 g | 10 g  
NLRP3 inflammasome inhibitor.

**LIT:** A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases: R.C. Coll, et al.; Nat. Med. **21**, 248 (2015)

### BAY 11-7082

AG-CR1-0013 10 mg | 50 mg  
NLRP3 inflammasome inhibitors, reducing ATPase activity of the NLRP3 inflammasome.

**LIT:** Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome: C. Juliana, et al.; J. Biol. Chem. **285**, 9792 (2010)

### Prostaglandin E2

AG-CL1-0001 1 mg | 5 mg  
inhibits the NLRP3 ATPase activity, which is required for assembly of NLRP3-ASC inflammasome complexes.

**LIT:** Prostaglandin E2 Inhibits NLRP3 Inflammasome Activation through EP4 Receptor and Intracellular Cyclic AMP in Human Macrophages: M. Sokolowska, et al.; J. Immunol. **194**, 5472 (2015)

### Parthenolide

AG-CN2-0455 10 mg | 50 mg | 250 mg  
NLRP3 inflammasome inhibitors, reducing ATPase activity of the NLRP3 inflammasome.

**LIT:** Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome: C. Juliana, et al.; J. Biol. Chem. **285**, 9792 (2010)

### Arglabin

AG-CN2-0458 1 mg | 5 mg  
NLRP3 inflammasome inhibitor.

**LIT:** Anti-Inflammatory and antiatherogenic effects of the NLRP3 Inflammasome inhibitor Arglabin in ApoE2.Ki mice fed a high-fat diet: A. Abderrazak, et al.; Circulation **131**, 1061 (2015)

### Vinpocetine

AG-CN2-0454 20 mg | 100 mg  
NLRP3 inflammasome inhibitor.

**LIT:** Vinpocetine inhibits amyloid-beta induced activation of NF-κB, NLRP3 inflammasome and cytokine production in retinal pigment epithelial cells: R.T. Liu, et al.; Exp. Eye Res. **127**, 49 (2014)

### 3-Hydroxybutyric acid

AG-CR1-3616 (R)-3-Hydroxybutyric acid 25 mg | 100 mg  
AG-CR1-3617 (S)-3-Hydroxybutyric acid 25 mg | 100 mg  
NLRP3 inflammasome inhibitors. Prevent K<sup>+</sup>-efflux and consequently reduce Asc oligomerization and speck formation.

**LIT:** The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease: Y.H. Youm, et al.; Nat. Med. **21**, 263 (2015)

### Resveratrol

AG-CN2-0033 50 mg | 100 mg | 500 mg  
NLRP3 inflammasome inhibitor.

**LIT:** Resveratrol inhibits NLRP3 inflammasome activation by preserving mitochondrial integrity and augmenting autophagy: Y.P. Chang, et al.; J. Cell Physiol. **230**, 1567 (2015)

### NEW K777 [K11777]

AG-CR1-0158 1 mg | 5 mg  
Broad-range cathepsin inhibitor useful for inflammasome inhibition.

**LIT:** Multiple cathepsins promote inflammasome-independent, particle-induced cell death during NLRP3-dependent IL-1β activation: G.M. Orłowski, et al.; J. Leukoc. Biol. **102**, 7 (2017)

UNIQUE

## ZBP1 – Innate Immune Sensor of Influenza A Virus

**NEW** anti-ZBP1, mAb (Zippy-1)

AG-20B-0010-C100

100 µg

**LIT:** ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways: T. Kuriakose, et al.; Sci. Immunol. 1, aag2045 (2016)

**Application:** ICC, IP, WB **Specificity:** Recognizes human and mouse ZBP1.

## Flagellin – NLRC4/NAIP5 Inflammasome Activators

THE SOURCE

Toll-like receptor 5 (TLR5) recognizes **flagellin** from both Gram-positive and Gram-negative bacteria. Activation of the receptor stimulates the production of proinflammatory cytokines, such as TNF- $\alpha$ , through signaling via the adapter proteins MyD88, TIRAP and TRIF. Flagellin is the subunit protein which polymerizes to form the filaments of bacterial flagella. It activates the innate immune system not only through the TLR5, but also through the intracellular NAIP5/NLRC4 (IPAF) inflammasome protein.

AdipoGen Life Sciences offers different types of **low endotoxin** and **high purity flagellins**, including pathway specific mutants. The Flagellin (NLRC4 Mutant) (rec.) (Prod. No. AG-40B-0126) is only detected by TLR5 not by NLRC4, whereas the Flagellin (TLR5 Mutant) (rec.) (Prod. No. AG-40B-0127) is only detected by NLRC4.

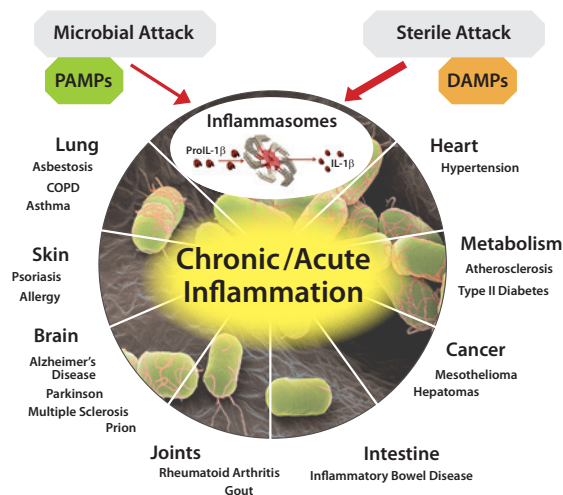
PRODUCT NAME	PID	SIZE
<b>Flagellin</b>	AG-40B-0095	100 µg
<b>Flagellin (high purity)</b>	AG-40B-0025	10 µg   3 x 10 µg
<b>Flagellin (rec.)</b>	AG-40B-0125	10 µg   3 x 10 µg
<b>NEW</b> <b>Flagellin (NLRC4 Mutant) (rec.)</b>	AG-40B-0126	10 µg   3 x 10 µg
<b>NEW</b> <b>Flagellin (TLR5 Mutant) (rec.)</b>	AG-40B-0127	10 µg   3 x 10 µg

## Inflammasomes – Therapeutic Implications

IL-1 $\beta$  is a key player in the inflammatory response, moving inflammatory caspases and inflammasomes in an important role in several diseases (see FIGURE). Several human hereditary or acquired diseases have been linked to elevated IL-1 $\beta$ , some of which can be treated by antagonists against IL-1 $\beta$  or its receptor. A number of diseases, known as cryopyrin-associated periodic syndromes (CAPS), have been directly linked to NLRP3 mutations.

Gout, an autoinflammatory disease characterized by severe joint inflammation, as well as the development of type 2 diabetes mellitus (T2DM) and insulin resistance are associated to elevated IL-1 $\beta$  levels. Thus by functioning as a sensor for metabolic stress, like in the form of monosodium urate (MSU) or hyperglycemia, the NLRP3 inflammasome likely contributes to the pathogenesis of gout or T2DM, respectively. In addition, several cancers have been associated to inflammasome-dependent inflammatory processes. Several inflammasome regulators (e.g. pyrin) were shown to have a significant relevance in diseases and may allow novel entry points for disease treatment.

**SELECTED LATEST REVIEW ARTICLES:** Inflammasome biology, molecular pathology and therapeutic implications: F. Awad, et al.; Pharmacol. Ther. (Epub ahead of print) (2018) • Current role of the NLRP3 inflammasome on obesity and insulin resistance: A systematic review: J. Rheinheimer, et al.; Metabolism 74, 1 (2017) • Inflammasomes: mechanism of action, role in disease, and therapeutics: H. Guo, et al.; Nat. Med. 21, 677 (2015)



**FIGURE:** Overview on inflammasome-associated diseases.