InvivoGen Insight
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Inflammasome activation: a matter of caspases

Inflammasomes are multimeric protein complexes that are crucial for host defense to infection and endogenous danger signals. They promote the secretion of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 and cause a rapid and pro-inflammatory form of cell death called pyroptosis. Inflammasomes assemble in the cytoplasm of innate immune cells, such as macrophages and dendritic cells, in response to cytosolic pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs).

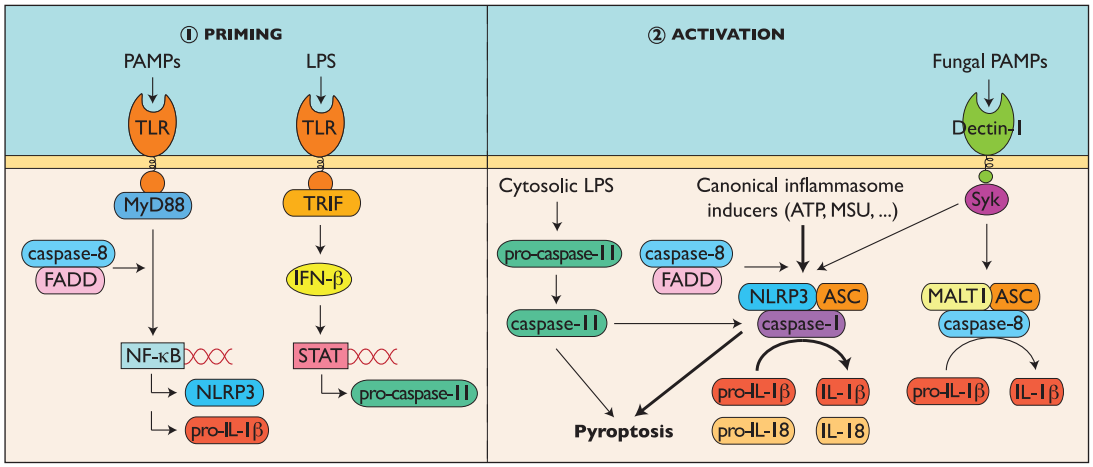
Several inflammasomes have been identified[1]. They generally contain an inflammasome sensor, the adaptor protein apoptosis-associated speck-like protein with a CARD (ASC) and pro-caspase-1. Typically, the inflammasome sensor is a member of the NOD-like receptor (NLR) or AIM2-like receptor (ALR) families. The most intensely studied inflammasome is the NLRP3 inflammasome. It is activated by a broad variety of stimuli, including danger signals (e.g. ATP), crystalline substances (e.g. MSU) and microbial toxins (e.g. nigericin). Once assembled, canonical inflammasomes catalyze the conversion of pro-caspase-1 into active caspase-1, which in turn proteolytically activates IL-1β and IL-18 and triggers pyroptosis. To avoid excessive inflammatory response, activation of the NLRP3 inflammasome is tightly regulated and requires a two-step process. The first step is the upregulation of NLRP3 and pro-IL-1β, which is induced by the detection of extracellular PAMPs by pattern recognition receptors (PRRs), such as LPS by Toll-like receptor (TLR) 4. The second step is the assembly of the inflammasome triggered upon detection of intracellular PAMPs/DAMPs.

Additional caspases, such as caspase-11 and caspase-8, have recently been identified as indispensable upstream mediators of caspase-1 in certain settings. In mice, infection by Gram-negative bacteria is detected by the NLRP3 inflammasome through a non-canonical pathway that requires caspase-11[2]. Detection of extracellular LPS by TLR4 induces the expression of pro-caspase-11 in a TRIF-dependent manner. Once upregulated, pro-caspase-11 becomes activated by intracellular LPS, independently of TLR4[3, 4]. A recent study suggests that cytosolic LPS binds directly to caspase-11 and human caspases-4 and -5 (the orthologs of murine caspase-11)[5]. Caspase-11 triggers pyroptosis and promotes the assembly of the NLRP3 inflammasome leading to caspase-1 activation. This is unique to NLRP3; all other inflammasome sensors activate caspase-1 independently of caspase-11.

Caspase-8, traditionally associated with the apoptotic pathway, has been shown to form a non-canonical inflammasome upon the detection of extracellular fungal or mycobacterial PAMPs by the PRR Dectin-1[6]. This caspase-8 inflammasome, which comprises MALT1, caspase-8 and ASC, directly processes pro-IL-1β into the bioactive cytokine, independently of caspase-1. Caspase-8 and its adaptor FADD have also been implicated in priming and activation of canonical as well as non-canonical NLRP3 inflammasomes[7].

Several canonical and non-canonical inflammasomes can form during infection and injury. They are differentially coordinated by caspase-1, caspase-8 and/or caspase-11 in function of the type of threat. Researchers are now tasked with elucidating the complex mechanisms of inflammasome activation. This work will prove indispensable for the development of targeted therapies for inflammatory diseases.

1. Latz E. *et al.*, 2013. Activation and regulation of the inflammasomes. Nat Rev Immunol. 13(6):397-411. **2. Kayagaki N. *et al.*, 2011.** Non-canonical inflammasome activation targets caspase-11. Nature. 479(7371):117-21. **3. Hagar JA. *et al.*, 2013.** Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science. 341(6151):1250-3. **4. Kayagaki N. et al., 2013.** Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science. 341(6151):1246-9. **5. Shi J. *et al.*, 2014.** Inflammatory caspases are innate immune receptors for intracellular LPS. Nature. 514(7521):187-92. **4. Gringhuis SI. *et al.*, 2012.** Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. Nat Immunol. 13(3):246-54. **7. Gurung P. *et al.*, 2014.** FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. J Immunol. 192(4):1835-46.



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Inflammasome Modulators

InvivoGen offers an expanding collection of molecules known to induce or inhibit activation of canonical or non-canonical inflammasomes, as well as TLR ligands commonly used to prime inflammasomes. All of these molecules are validated for purity and activity using our in-house inflammasome assay based on inflammasome test cells, which are THP1-derived, and our IL-1β reporter cell line, HEK-Blue IL-1β.

➤ Inflammasome Inducers

PRODUCT	TARGET	QTY	CAT. CODE
Canonical Inflammasome Inducers			
Alum Crystals	NLRP3	1g	tlrl-alk
ATP	NLRP3	1g	tlrl-atp
Chitosan Ultrapure NEW	NLRP3	100 mg	tlrl-cht
Chitosan VacciGrade™ NEW	NLRP3	100 mg	vac-cht
CPPD Crystals	NLRP3	5 mg	tlrl-cppd
FLA-PA (P. *aeruginosa* flagellin)	NLRC4	50 µg	tlrl-pafla
FLA-ST (S. *typhimurium* flagellin)	NLRC4	10 µg	tlrl-epstfla
Hemozoin	NLRP3	5 mg	tlrl-hz
HKMT	NLRP3	10 mg	tlrl-hkmt-1
L18-MDP	NLRP1	1 mg	tlrl-lmdp
MDP	NLRP1	5 mg	tlrl-mdp
MSU Crystals	NLRP3	5 mg	tlrl-msu
Nano-SiO2	NLRP3	10 mg	tlrl-sio
Nigericin	NLRP3	10 mg	tlrl-nig
Poly(dA:dT)	AIM2	200 µg	tlrl-patn
TDB	NLRP3	2x 1 mg	tlrl-tdb
Non-canonical Inflammasome Inducers			
Curdlan AL	Dectin-1/Caspase-8	1 g	tlrl-cura
HKCA	Dectin-1/Caspase-8	10^9 cells	tlrl-hkca
Pustulan	Dectin-1/Caspase-8	100 mg	tlrl-pst
Zymosan Depleted	Dectin-1/Caspase-8	10 mg	tlrl-dzn

➤ Inflammasome Inhibitors

PRODUCT	TARGET	QTY	CAT. CODE
Bay 11-7082	NLRP3	10 mg	tlrl-b82
Glybenclamide	NLRP3	1 g	tlrl-gly
Isoliquiritigenin NEW	NLRP3	10 mg	inh-ilg
Parthenolide NEW	Caspase-1 & NLRP3	50 mg	inh-ptd
VX-765	Caspase-1	10 mg	inh-vx765-1
Z-VAD-FMK	Pan-caspase	1 mg	tlrl-vad

➤ Inflammasome Primers

PRODUCT	TARGET	QTY	CAT. CODE
LPS-EB Ultrapure	TLR4 agonist	5x 10^6 EU	tlrl-3pelps
Pam3CSK4	TLR2 agonist	1 mg	tlrl-pms
Poly(I:C)	TLR3 agonist	10 mg	tlrl-pic

For more information, visit www.invivogen.com/inflammasome

Chitosan

NLRP3 inflammasome inducer & vaccine adjuvant

Chitosan is the deacylated derivative of chitin, an abundant polysaccharide found in fungal cell walls, crustacean shells, and insect exoskeletons. This natural polymer is non-toxic and has high bioavailability, charge density and mucoadhesivity, a unique combination of properties that makes chitosan an attractive carrier for the delivery of drugs[1] and genes[2]. In addition, chitosan is immunostimulatory: it activates the NLRP3 inflammasome, leading to potent IL-1β production[3] and thus is of great interest as an adjuvant for mucosal vaccination[4].

InvivoGen provides an ultra-pure chitosan of non-animal origin, purified from white edible mushrooms (*Agaricus bisporus*).

Two grades are available:

- Chitosan Ultrapure
- Chitosan VacciGrade™

Both products have a molecular weight of 60 to 120 kDa and a degree of acetylation ranging from 15 to 25 %, and have been formulated to increase their solubility. In addition, Chitosan VacciGrade™ is endotoxin-free (< 5 EU/mg) and guaranteed to be sterile.

Isoliquiritigenin

NLRP3 inflammasome inhibitor

Isoliquiritigenin (ILG), a simple chalcone-type flavonoid isolated from licorice root (*Glycyrrhiza uralensis*), exhibits anti-oxidant, anti-inflammatory, and anti-tumor activities[5]. ILG was recently reported to block LPS-induced TLR4/MD2 complex signaling and NF-κB activation[6], and to inhibit NLRP3-activated ASC oligomerization[7]. Interestingly, NLRP3-dependent IL-1β production has been inhibited with low concentrations of ILG (1 to 10 µM). Thus, ILG can block the NLRP3 inflammasome at both the priming step and the activation step.

Parthenolide

Caspase-1 and inflammasome inhibitor

Parthenolide, a sesquiterpene lactone derived from feverfew, is a known inhibitor of NF-κB activation. It is also a direct inhibitor of caspase-1 and consequently of multiple inflammasomes, including the NLRP3 and NLRP1 inflammasomes[8]. Parthenolide has been found to block caspase-1 activation by alkylation of the p20 subunit. Further, parthenolide directly inhibits the NLRP3 inflammasome by interfering with NLRP3 ATPase activity.

1. Garcia-Fuentes M1 & Alonso MJ., 2012. Chitosan-based drug nanocarriers: where do we stand? J Control Release. 161(2):496-504. **2. Tong H. *et al.*, 2009.** Progress and prospects of chitosan and its derivatives as non-viral gene vectors in gene therapy. Curr Gene Ther. 9(6):495-502. **3. Bueter CL. *et al.*, 2014.** Spectrum and mechanisms of inflammasome activation by chitosan. J Immunol. 192(12):5943-51. **4. Islam MA. *et al.*, 2012.** Design and application of chitosan microspheres as oral and nasal vaccine carriers: an updated review. Int J Nanomedicine. 6077-93. **5. Jung SK. *et al.*, 2014.** Isoliquiritigenin Induces Apoptosis and Inhibits Xenograft Tumor Growth of Human Lung Cancer Cells by Targeting Both Wildtype and L858R/T790M Mutant EGFR. J Biol Chem. 2014 [Ahead of print]. **6. Honda H. *et al.*, 2012.** Glycyrrhizin and isoliquiritigenin suppress the LPS sensor toll-like receptor 4/MD-2 complex signaling in a different manner. J Leukoc Biol. 91(6):967-76. **7. Honda H. *et al.*, 2014.** Isoliquiritigenin is a potent inhibitor of NLRP3 inflammasome activation and diet-induced adipose tissue inflammation. J Leukoc Biol. 96(6):1087-100. **8. Juliana C. *et al.*, 2010.** Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome. J Biol Chem. 285(13):9792-802.

Inflammasome Test Cells

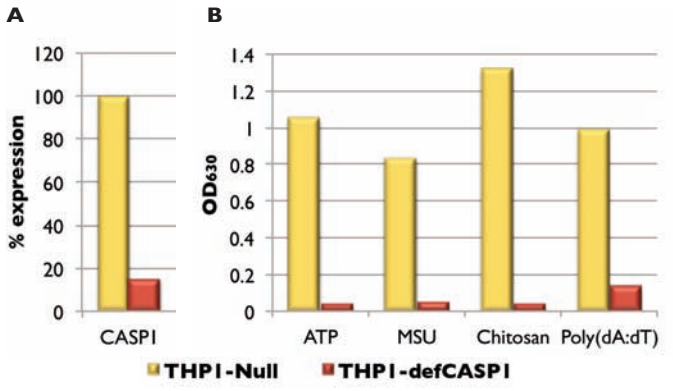
THP-1 human monocytic cells are the most commonly used model cell line for studying inflammasome activation. They express high levels of NLRP3, ASC and pro-caspase-1. InvivoGen provides engineered THP-1 cells that express reduced levels of NLRP3 or ASC and we are now introducing a new CASP-1 knockdown cell line. All of these cell lines are useful for determining whether a signal activates the inflammasome, in particular the NLRP3 inflammasome.

THP1-defCASP1

Caspase-1 deficient THP-1 cells

THP1-defCASP1 cells are highly deficient for caspase-1 activity (~7 fold reduction). Compared to their parental cell line THP1-Null, they produce significantly less IL-1β in response to stimuli that activate the inflammasomes, such as ATP, MSU, and chitosan (NLRP3 inflammasome) or transfected poly(dA:dT) (AIM2 inflammasome).
THP1-defCASP1 cells and THP1-Null cells together enable you to determine whether a compound is an inflammasome inducer. Production of IL-1β can be detected using InvivoGen's HEK-Blue IL-1β reporter cell line or our recently launched LumiKine™ IL-1 ELISA kits.

PRODUCT	QTY	CAT. CODE
THP1-defCASP1 NEW	3-7 x 10^6 cells	thp-dcasp1
THP1-defASC	3-7 x 10^6 cells	thp-dasc
THP1-defNLRP3	3-7 x 10^6 cells	thp-dnlp
THP1-Null	3-7 x 10^6 cells	thp-null
HEK-Blue™ IL-1β	3-7 x 10^6 cells	hkb-il1b



Expression of CASP1 determined by quantitative RT-PCR (A) and IL-1β production in THP1-Null and THP1-defCASP1 cells (B). For graph B, THP1-null and THP1-defCASP1 cells were primed with LPS (1 μg/ml) then stimulated with ATP (5 mM), MSU (100 μg/ml), Chitosan Ultrapure (100 μg/ml) or transfected poly(dA:dT) (0.5 μg/ml). After 24h incubation, the supernatants were added to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using the QUANTI-Blue™ assay.

IL-1 Detection with LumiKine™ - Bioluminescent ELISA kits NEW

LumiKine™ Xpress is InvivoGen's new immunoassay for rapid, sensitive and specific detection of cytokines. By replacing the absorbance-based enzymatic detection reaction of a standard ELISA with its lightning-fast Lucia luciferase/QUANTI-Luc™ bioluminescence detection system, InvivoGen has created an assay that can save you precious time in the laboratory. Unlike many ELISA kits, which are based on *E. coli*-produced cytokines, LumiKine™ has been designed using cytokines produced in mammalian cells, for accurate detection of species-specific mammalian cytokines. Furthermore, it allows you to detect the cytokine of your interest in a broad concentration range that makes quantification and calibration curves a snap.

- **Rapid -** Optimized to reduce assay time
- **Specific -** Antibodies raised against natural cytokines
- **Precise -** Wide concentration range
- **Economical -** Each kit contains enough reagents to run five ELISA plates

LumiKine™ Xpress IL-1 Kits

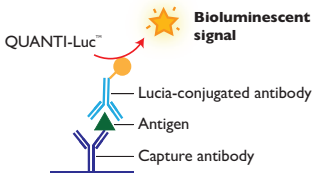
LumiKine™ Xpress IL-1 kits are bioluminescent ELISA kits designed to quantify the levels of interleukin-1 (IL-1) cytokines (IL-1α or IL-1β) in cell culture supernatant, serum and plasma samples. These kits contain recombinant standards produced in mammalian cells and antibodies raised against the natural proteins by DNA immunization. The enzyme used for detection is Lucia luciferase. It is directly fused to the detection antibody, eliminating the biotinylated antibody-streptavidin conjugate incubation step. Addition of QUANTI-Luc™, which contains the luciferase substrate, instantaneously generates a bioluminescent signal that is directly proportional to the concentration of the cytokine. This signal is quantified by luminometry.

Also Available

LumiKine™ Cytokine Kits contain biotinylated secondary antibodies and a streptavidin-Lucia conjugate for detection. You can replace this conjugate with the streptavidin conjugate of your choice.

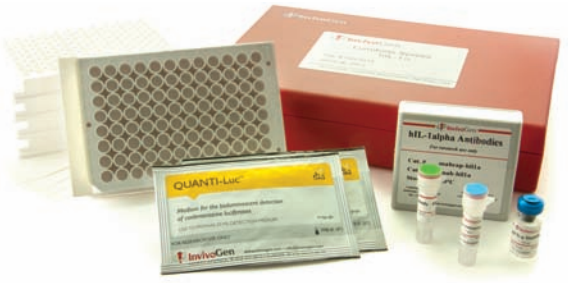
KIT CONTENTS

- Capture antibody
- Lucia-conjugated antibody
- Recombinant cytokine
- QUANTI-Luc™
- White immunoplates



PRODUCT	QTY	CAT. CODE
LumiKine™ Xpress hIL-1α (human)	5 plates	luex-hil1a
LumiKine™ Xpress hIL-1β (human)	5 plates	luex-hil1b
LumiKine™ Xpress mIL-1β (mouse)	5 plates	luex-mil1b

For more information, visit www.invivogen.com/cytokine-elisa



Microbial Contamination

Be vigilant to keep your cells safe

Microbial contamination of cell cultures is a constant and serious threat to your research. Invasive mycoplasmas, bacteria, yeast and fungi can kill or drastically alter your precious cell cultures, leading to erroneous results, lost time and wasted resources. InvivoGen offers a wide range of highly specific antimicrobial agents to help you prevent, detect and eliminate microbial contamination.

Prevention

To prevent microbial contamination, make sure to work in sterile conditions, use proper aseptic technique and regularly monitor incoming cell cultures and culture reagents for microbes. In special cases, such as seeding of primary cells, infection can be prevented through judicious use of antimicrobial agents in the culture medium.

- **Plasmocin™ Prophylactic -** Prevention of mycoplasmas
- **Normocin™ -** Prevention of mycoplasmal, bacterial and fungal infections
- **Primocin™ -** Prevention of mycoplasmal, bacterial and fungal infections in primary cells

Detection

Microbial contamination must be detected as early as possible. The presence of bacteria or fungi can usually be determined by the naked eye: contaminated cultures will appear turbid or spotty. Optical microscopy, biological assays (e.g. PCR), physicochemical measurements (e.g. pH) or other methods (e.g. fluorescence staining) can then be used for confirmation. However, mycoplasma in cell cultures cannot be detected visually, not even by optical microscopy; hence, they can go unnoticed for long periods and can only be identified using dedicated tests. Regardless of the suspected microbe, use of complimentary detection techniques is preferable, especially to rule out false positives or clarify ambiguous results.

- **PlasmoTest™ -** Detection of mycoplasma contamination

Elimination

Upon the first sign of contamination, you should immediately quarantine and treat any affected cells to save them and to stop the contamination from spreading. Each type of microbe affects cells differently and thus, must be addressed specifically.

- **Plasmocin™ Treatment -** Treatment for mycoplasma contamination
- **Plasmocure™ -** Alternative treatment for mycoplasma contamination
- **Fungin™ -** Elimination and prevention of yeast and fungal infections
- **Normocure™ -** Elimination of bacterial infections

Normocure™

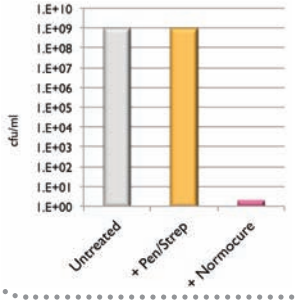
InvivoGen's latest antimicrobial agent

Normocure™ is a broad-spectrum antibiotic that is highly effective against Gram-positive and Gram-negative bacteria. Cell cultures contaminated with bacteria from the environment, such as *Pseudomonas* species[1], *Staphylococcus* species[2] and *Achromobacter* species[3], can be efficiently cured by Normocure™ treatment. Unlike most antibiotics used to treat cell cultures, such as Penicillin-Streptomycin, Normocure™ is also active against most multidrug resistant bacteria.

Normocure™ contains three bactericidal components belonging to different antibiotic families. They act by inhibiting protein synthesis or disrupting membrane integrity. They have different targets, each of which is completely absent in eukaryotic cells.

Normocure™ is provided as a ready-to-use solution (50 mg/ml). Simply add it to bacteria contaminated cell cultures at the recommended concentration (100 µg/ml) for 2 weeks. After three passages, the bacterial contamination is totally eliminated.

1. Jorgen Fogh, 1973. Contamination in Tissue Culture, published by Academic Press Inc. **2. Mirjalili A, *et al.*, 2005.** Microbial contamination of cell cultures: a 2 years study. Biologicals. 33(2):81-85. **3. Gray JS. *et al.*, 2010.** Got black swimming dots in your cell culture? Identification of Achromobacter as a novel cell culture contaminant. Biologicals. 38(2):273-7.



HEK293 cells (3×10^5 cells/ml) were spiked with a mixture of Gram⁻ non-fermenting bacilli (*Pseudomonas aeruginosa*, *Alcaligenes xylosodans*, *Achromobacter sp.* and *Stenotrophomonas maltophilia*) at the concentration of 10^5 colony forming units (cfu)/ml, and were then either left untreated, or treated with 100 U/ml penicillin and 100 µg/ml streptomycin, or with 100 µg/ml Normocure™. After 4 days at 37°C, 5% CO_2, the bacteria were quantified (cfu per ml).

PRODUCT	QTY	CAT. CODE
Fungin™	7.5 ml (200X)	ant-fn-1
Normocin™	10 ml (500X)	ant-nr-1
Normocure™	2 ml (500X)	ant-noc
Plasmocin™ Prophylactic	10 ml (500X)	ant-mpp
Plasmocin™ Treatment	10 ml (1000X)	ant-mpt
Plasmocure™	1 ml (2000X)	ant-pc
PlasmoTest™	1 kit (250 samples)	rep-pt1
Primocin™	10 ml (500X)	ant-pm-1

For more information, visit
www.invivogen.com/cell-culture-contamination

InvivoGen