

2<sup>nd</sup> Edition

# Innate Immunity

## Focus Toll-like Receptor (TLR) Agonists

The innate immune system plays an essential role in the host's first line of defense against microbial invasion and involves the recognition of pathogen-associated molecular patterns (**PAMPs**) or endogenous danger signals through the sensing of danger-associated molecular patterns (**DAMPs**) by pattern recognition receptors (PRRs). Activation of PRRs triggers cell signaling leading to the production of proinflammatory cytokines, chemokines and type 1 interferons, the induction of antimicrobial and inflammatory responses, pyroptotic cell death and the recruitment of phagocytic cells. These innate responses are responsible for efficient destruction and clearance of invading pathogens and other molecular threats and instructing the development of an appropriate pathogen-specific adaptive immune response.

The innate immune system comprises several classes of PRRs that allow the early detection of pathogens at the site of infection. The membrane-bound **Toll-like receptors (TLRs)** and **C-type lectin receptors (CLRs)** detect PAMPs in extracellular milieu and endosomal compartments. TLRs and CLRs cooperate with PRRs sensing the presence of cytosolic nucleic acids, like RNA-sensing **RIG-I (retinoic acid-inducible gene I)-like receptors** (RLRs; RLHs) or the DNA-sensing AIM2. Another set of intracellular sensing PRRs are the **NOD-like receptors** (NLRs; nucleotide-binding domain leucine-rich repeat containing receptors), which not only recognize PAMPs but also DAMPs. Upon stress (including infections and metabolic deregulation), certain NLRs form high molecular weight complexes called **inflammasomes**. These complexes and the self-degrading process autophagy play central roles in controlling innate and adaptive immunity (see Wallchart next Page).

### Toll-like receptors (TLRs)

Toll-like receptors (TLRs) are a family of evolutionally conserved pattern recognition receptors (PRRs) expressed by a variety of cell types, particularly those of the innate immune system. TLRs are type I membrane glycoproteins, characterized by a cytoplasmic TIR (Toll/interleukin-1 receptor (IL-1R)) domain and a leucine-rich repeat domain. They are capable of detecting exogenous **PAMPs** such as lipopolysaccharide (LPS), lipopeptides, flagellin, bacterial DNA and viral dsRNA, as well as endogenous, host-derived DAMPs, including HMGB1 and  $\beta$ -defensins.

The activation of TLR signaling pathways results in the production and release of various cytokines and chemokines. TLRs play a crucial role in host defence and inflammation and are implicated in the pathogenesis of immune diseases and cancer. TLR agonists are being tested as vaccines, enhancing tumor immunity by targeting immune checkpoints or inducing the expansion of T cells by potent adjuvants. Therefore, TLR agonists can activate both the innate and adaptive immune systems, play an important role in antiviral and antitumor immunity and are exploited as potent adjuvants to enhance tumor immunity.

**SELECTED REVIEW ARTICLES** The Role of Toll-Like Receptor in Inflammation and Tumor Immunity: X. Cen, et al.; Front. Pharmacol. **9**, 878 (2018) • Toll-like receptors: Activation, signalling and transcriptional modulation: D. De Nardo; Cytokine **74**, 181 (2015) • Characterization of innate immune signalings stimulated by ligands for pattern recognition receptors: T. Kameyama & A. Takaoka; Methods Mol. Biol. **1142**, 19 (2014)

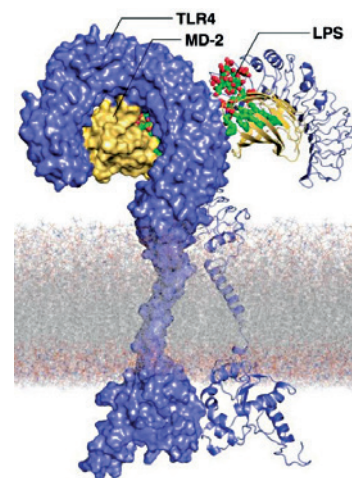


FIGURE 1: TLR4/MD-2/LPS complex.

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> See Inflammasome Brochure!





## Selected TLR Agonists & STING Ligand

### TLR1/2 Complex Agonist

#### Pam<sub>3</sub>Cys-Ser-(Lys)<sub>4</sub> · 3HCl

AG-CP3-0003

2 mg

### TLR3/MDA5 Agonist

#### Poly(I:C) (Endotoxin-free) (sterile)

IAX-200-021

2 mg | 5 mg | 3 x 5 mg | 5 x 5 mg

### TLR11/TLR12 Agonist

#### Profilin (*Toxoplasma gondii*) (rec.)

AG-40B-0121

10 µg | 3 x 10 µg

### STING Activator

#### cGAMP · 2Na

AG-CR1-3588

100 µg | 500 µg

## FSL-1 – The MALP-2 Alternative

### TLR6/TLR2 Agonists

#### (R)-FSL-1

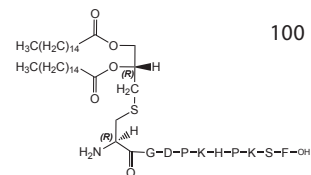
AG-CP3-0010

100 µg

Formula: C<sub>84</sub>H<sub>140</sub>N<sub>14</sub>O<sub>18</sub>S

MW: 1666.2

CAS: 322455-70-9 (R/S)



Potent stimulator of TLR2/TLR6. The naturally occurring R-stereoisomer is biologically more active than the S-stereoisomer.

#### FSL-1

AG-CP3-0009

100 µg

## TLR5/TLR11/NLRC4 Agonist – Flagellin & Mutants

Toll-like receptor 5 (TLR5) recognizes **flagellin**, the principal component of bacterial flagella, 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 activates the innate immune system not only through the TLR5, but also through the intracellular NAIP5/NLRC4 (IPAF) inflammasome protein. In mice, Flagellin is also recognized by the cathepsin-cleaved TLR11, which is evolutionarily the closest member of the TLR5 gene family.

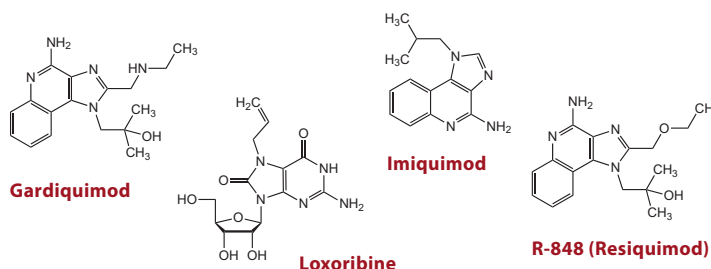
AdipoGen Life Sciences offers different types of **low endotoxin** and **high purity flagellins**, including **pathway specific mutants**. The Flagellin (NLRC4 Mutant) (rec.) (Prod. No. AG-40B-0126) is only detected by TLR5 but not by NLRC4, whereas the Flagellin (TLR5 Mutant) (rec.) (Prod. No. AG-40B-0127) 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> Flagellin (NLRC4 Mutant) (rec.)	AG-40B-0126	10 µg   3 x 10 µg
<b>NEW</b> Flagellin (TLR5 Mutant) (rec.)	AG-40B-0127	10 µg   3 x 10 µg

## TLR7/TLR8 Agonists

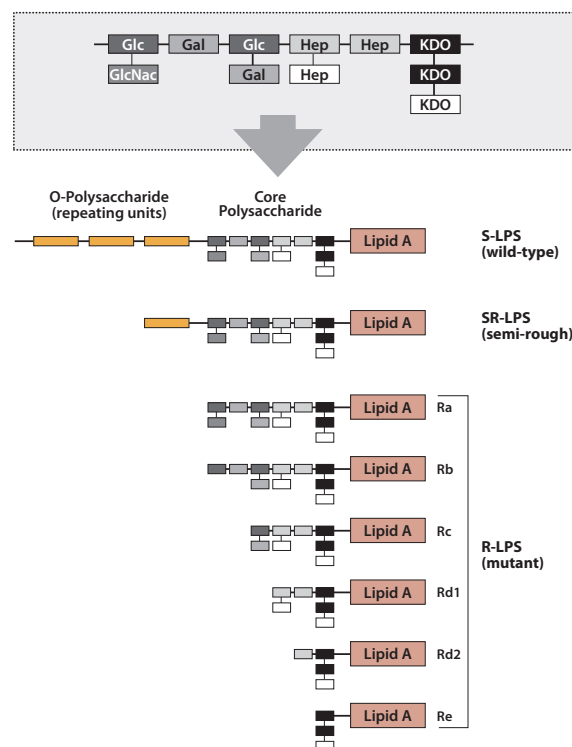
Toll-like receptor 7 (TLR7) and TLR8 play an important role in the immune response to viral infection. They recognize single stranded RNAs as their natural ligand and also small synthetic molecules such as imidazoquinolines\* and nucleoside\*\* analogs. The TLR7 agonist Imiquimod can also contribute to NLRP3 activation through the inhibition of mitochondria complex I and through endolysosomal effects.

PRODUCT NAME	PID	SIZE
<b>TLR7 Agonists</b>		
<b>Gardiquimod *</b>	AG-CR1-3583	5 mg   25 mg
<b>Imiquimod *</b>	AG-CR1-3569	100 mg   250 mg
<b>Loxoribine **</b>	AG-CR1-3584	5 mg   25 mg
<b>TLR7/8 Agonist &amp; NLRP3 Activator</b>		
<b>R-848 (Resiquimod)*</b>	AG-CR1-3582	5 mg   25 mg



# TLR4 Agonists – LPS, Lipid A and MPLA

Bacterial lipopolysaccharide (LPS) is the major structural component of the outer wall of all Gram-negative bacteria and a potent activator of the immune system. Activation of cells by LPS is mediated by the extracellular receptor Toll-like receptor 4 (TLR4) or by intracellular recognition and activation of caspase-11 (caspase-4/5 in humans) depending on extracellular or intracellular triggers, respectively. For optimal interaction with LPS, TLR4 requires association with myeloid differentiation protein 2 (MD-2). According to current consensus activation of TLR4 is preceded by the transfer of LPS to membrane-bound (m) or soluble (s) CD14 by LPS-binding protein (LBP). R-form LPS and lipid A, but not S-form LPS, are capable of inducing TNF- $\alpha$  responses also in the absence of CD14. LPS, synthesized by most wild-type (WT) Gram-negative bacteria (S-form LPS), consists of three regions, the O-polysaccharide chain, which is made up of repeating oligosaccharide units, the core oligosaccharide and the lipid A, which harbors the endotoxic activity of the entire molecule (see Figure 2). R-form LPS synthesized by the so-called rough (R) mutants of Gram-negative bacteria lacks the O-specific chain. The core oligosaccharide may be present in different degrees of completion, depending on the class (Ra to Re) to which the mutant belongs. LPS are amphipathic molecules whose hydrophobicity decreases with increasing length of the sugar part. Based upon these differences, S- and R-form LPS show marked differences in the kinetics of their blood clearance and cellular uptake as well as in the ability to induce oxidative burst in human granulocytes and to activate the host complement system. **S-form LPS is the preferred *in vivo* TLR4 agonist, whereas R-form LPS can activate TLR4 on a variety of cells *in vitro*, which do not express CD14 or where the cell culture medium does not contain sufficient quantities of soluble CD14 or LBP.**



**FIGURE 2:** Schematic representation of the different LPS chemotypes: GlcNAc = N-Acetylglucosamine; Glc = Glucose; Gal = Galactose; Hep = Heptose; KDO = 2-Keto-3-desoxyoctonate. Adapted from M. Huber, et al.; Eur. J. Immunol. 36, 701 (2006).

**LIT:** CD14 is required for MyD88-independent LPS signaling: Z. Jiang, et al.; Nat. Immunol. 6, 565 (2005) • R-form LPS, the master key to the activation of TLR4/MD-2-positive cells: M. Huber, et al.; Eur. J. Immunol. 36, 701 (2006)

## TLRpure™ LPS from Innaxon – The TLR4/LPS Experts – Highest Quality

- **TLRpure™:** Qualified Purity & Activity
- **High potency TLR4-specific ligands**
- **Ultrapur** (no detectable protein, RNA & DNA)
- **Standardized aqueous sterile solutions**
- **Tested on TLR4 KO murine macrophages**
- **BULK available for *in vivo* studies**
- **Excellent lot-to-lot reproducibility**
- **Broadest LPS chemotype and serotype selection**



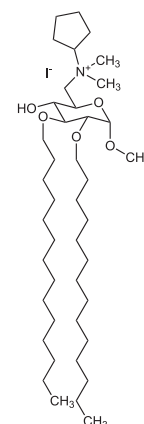
**UNIQUE**

## IAXO Compounds – Inhibitors of Sterile Inflammation

The novel IAXO compounds are synthetic TLR4/CD14 ligands with TLR4 modulating activities *in vitro* and conferring protection against TLR4/CD14-mediated tissue damage and inflammation *in vivo* [1-3]. IAXOs are useful to explore CD14-dependent and TLR4-independent pathways and TLR4 activation by endogenous ligands (e.g. hyaluronic acid oligosaccharides, oxLDL, HMGB1) in sterile inflammation. IAXO compounds have been shown to inhibit neuropathic pain [1], secondary necrosis of acute drug-induced liver failure [2] and vascular inflammation and abdominal aortic aneurysm [3] by blocking non-hematopoietic TLR4 signaling. They are useful tools, where inhibition of sterile (auto-) inflammation is desired, without compromising TLR4's key role in the defense of pathogens.

**LIT:** [1] I. Bettoni, et al.; Glia 56, 1312 (2008) • [2] N. Shah, et al.; Gut 61, A28 (2012) • [3] C. Huggins, et al.; Atherosclerosis 241, e53 (2015)

PRODUCT NAME	PID	SIZE
<b>IAXO-101 (CD14/TLR4 Antagonist) (synthetic)</b>	IAX-600-001	1 mg   5 mg
<b>IAXO-102 (CD14/TLR4 Antagonist) (synthetic)</b>	IAX-600-002	1 mg   5 mg
<b>IAXO-103 (CD14/TLR4 Antagonist) (synthetic)</b>	IAX-600-003	1 mg   5 mg



## TLR4 Agonists: Homogenous Ready-to-Use LPS Solutions

**UNIQUE**

PRODUCT NAME	PID	SIZE
<b>S-form LPS</b>		
LPS from <i>E. coli</i> O8:K27 (S-form) TLRpure™ Sterile Solution	IAX-100-006	500 µg   1 mg   5 x 1 mg
LPS from <i>E. coli</i> O111:B4 (S-form) TLRpure™ Sterile Solution	IAX-100-012	500 µg   1 mg   5 x 1 mg
LPS from <i>E. coli</i> O55:B5 (S-form) TLRpure™ Sterile Solution	IAX-100-013	500 µg   1 mg   5 x 1 mg
<b>THE STANDARD</b> LPS (Universal)* from <i>S. abortus equi</i> (S-form) TLRpure™ Sterile Solution	IAX-100-009	500 µg   1 mg   5 x 1 mg
LPS from <i>S. enteritidis</i> (S-form) TLRpure™ Sterile Solution	IAX-100-019	500 µg   1 mg
LPS from <i>S. minnesota</i> (S-form) TLRpure™ Sterile Solution	IAX-100-020	500 µg   1 mg   5 x 1 mg
LPS from <i>S. typhimurium</i> (S-form) TLRpure™ Sterile Solution	IAX-100-011	500 µg   1 mg   5 x 1 mg
<b>R-form LPS</b>		
LPS from <i>E. coli</i> EH100 (Ra) TLRpure™ Sterile Solution	IAX-100-010	500 µg   1 mg   5 x 1 mg
LPS from <i>E. coli</i> J5 (Rc) TLRpure™ Sterile Solution	IAX-100-014	500 µg   1 mg   5 x 1 mg
<b>THE STANDARD</b> LPS from <i>E. coli</i> R515 (Re) TLRpure™ Sterile Solution	IAX-100-007	500 µg   1 mg   5 x 1 mg
<b>THE STANDARD</b> LPS from <i>S. minnesota</i> R595 (Re) TLRpure™ Sterile Solution	IAX-100-008	500 µg   1 mg   5 x 1 mg
LPS from <i>S. minnesota</i> R345 (Rb) TLRpure™ Sterile Solution	IAX-100-015	500 µg   1 mg   5 x 1 mg
LPS from <i>S. minnesota</i> R60 (Ra) TLRpure™ Sterile Solution	IAX-100-016	500 µg   1 mg
LPS from <i>S. minnesota</i> R5 (Rc) TLRpure™ Sterile Solution	IAX-100-017	500 µg   1 mg   5 x 1 mg
LPS from <i>S. minnesota</i> R7 (Rd1) TLRpure™ Sterile Solution	IAX-100-018	500 µg   1 mg   5 x 1 mg
LPS from <i>S. minnesota</i> R3 (Rd2) TLRpure™ Sterile Solution	IAX-100-021	500 µg   1 mg   5 x 1 mg
<b>Lipid A und MPLA</b>		
Lipid A from <i>E. coli</i> R515 (Re) TLRpure™ Sterile Solution	IAX-100-004	250 µg   500 µg   1 mg
Lipid A from <i>S. minnesota</i> R595 (Re) TLRpure™ Sterile Solution	IAX-100-001	250 µg   500 µg   1 mg
MPLA from <i>E. coli</i> R515 (Re) TLRpure™ Sterile Solution	IAX-100-003	250 µg   500 µg   1 mg
MPLA from <i>S. minnesota</i> R595 (Re) TLRpure™ Sterile Solution	IAX-100-002	250 µg   500 µg   1 mg

\* Universal: High-stability LPS solution with long-term consistent high TLR4-exclusive potency in immune activation.

## Synthetic TLR4 Agonists – Kdo2-Lipid A and MPLA

PRODUCT NAME	PID	SIZE
<b>Kdo2-Lipid A (ready-to-use)</b>	AG-CU1-0001	1 mg
<b>MPLA (synthetic) Sterile Solution</b>	AG-CU1-0002	100 µg

## TLR4 Signaling Pathway Regulatory Proteins

High mobility group box 1 (HMGB1) is a Damage-Associated Molecular Pattern (DAMP) that interacts with multiple pattern recognition receptors (PRRs). Many of its effects in sterile inflammation models occur through an interaction with the TLR4/myeloid differentiation protein 2 (MD-2) complex. SIGIRR negatively regulates TLR4 signaling pathways by interacting transiently with TLR4, IRAK4 and TRAF6. The ST2 receptor, a member of the Toll/IL-1 receptor superfamily, has been shown to function as a negative regulator of TLR4 and TLR2 signaling by sequestering MAL and MyD88. CD14 acts as a co-receptor with TLR4/MD-2 for the detection of bacterial lipopolysaccharide. CD36 is a scavenger receptor shown to promote sterile inflammation through assembly of a TLR4/TLR6 heterodimer upon binding to oxLDL or β-Amyloid.

PROTEINS	PID
<b>HMGB1 (human) (rec.) (His)</b>	CHI-HR-200HMGB1
<b>HMGB1 (rat) (rec.) (His)</b>	CHI-RR-300HMGB1
<b>HMGB1 (rat):Fc (human) (rec.)</b>	CHI-RF-311HMGB1
<b>SIGIRR (human):Fc (human) (rec.)</b>	AG-40A-0093T
<b>ST2 (human):Fc (human) (rec.)</b>	AG-40A-0059
<b>IL-33R (human):Fc (human) (rec.)</b>	CHI-HF-21033R
<b>IL-33R (mouse):Fc (mouse) (rec.)</b>	CHI-MF-11033R

PROTEINS	PID
<b>anti-CD14 (human), mAb (UCHM1)</b>	ANC-163-020
<b>anti-CD36 (human), mAb (SMO)</b>	ANC-185-020
<b>anti-ST2 (human), pAb</b>	AG-25A-0058
<b>anti-ST2 (human), pAb (ATTO 488)</b>	AG-25A-0058YTD
<b>anti-ST2 (human), pAb (ATTO 647N)</b>	AG-25A-0058YTS
<b>anti-ST2 (human), mAb (ST33868)</b>	AG-20A-0044
<b>anti-HMGB1, mAb (rec.) (Giby-1-4)</b>	AG-27B-0002

# TLR9 Agonists – Unmethylated CpG Dinucleotides [ODNs]

Unmethylated CpG dinucleotides within particular sequence contexts are responsible for the immunostimulatory activity of bacterial DNA. Synthetic oligonucleotides (ODN), that contain such CpG motifs (CpG ODNs), mimic microbial DNA and are detected by Toll-like receptor 9 (TLR9).

## Endotoxin-free & Sterile ODNs from Innaxon

- TLR<sup>pure</sup><sup>™</sup>: Qualified Purity & Activity
- Endotoxin-free and sterile
- Potent TLR9 ligands
- Tested on TLR9 KO murine macrophages
- BULK available for *in vivo* studies (pre-clinical grade)
- ddWater Endotoxin-free (sterile) included



## Stimulatory ODNs (CpG ODNs)

Different types of CpG ODNs were identified based on their biological effects on different cell types through TLR9: **ODN Type A** is a potent inducer of IFN- $\alpha$  in human PDC, leading to antigen presenting cell (APC) maturation, whereas **ODN Type B** is a weak inducer of IFN- $\alpha$  but rather stimulates IL-8 production and increasing costimulatory and Ag-presenting molecules and triggers proliferation of B cells and IL-6 production. A third type of CpG ODN, termed **ODN Type C**, shows high induction of INF- $\alpha$  in PDC and activation of B cells.

PRODUCT NAME	PID	SIZE
<b>ODN 1668 (Type B) Endotoxin-free (sterile)</b>	IAX-200-001	100 $\mu$ g   1 mg   3 x 1 mg
<b>THE STANDARD ODN 1826 (Type B) Endotoxin-free (sterile)</b>	IAX-200-002	100 $\mu$ g   1 mg   3 x 1 mg
<b>THE STANDARD ODN 2216 (Type A) Endotoxin-free (sterile)</b>	IAX-200-005	100 $\mu$ g   1 mg   3 x 1 mg
<b>ODN 1585 (Type A) Endotoxin-free (sterile)</b>	IAX-200-003	100 $\mu$ g   1 mg   3 x 1 mg
<b>ODN M362 (Type C) Endotoxin-free (sterile)</b>	IAX-200-004	100 $\mu$ g   1 mg
<b>ODN 2006 (Type B) Endotoxin-free (sterile)</b>	IAX-200-006	100 $\mu$ g   1 mg   3 x 1 mg
<b>ODN 2395 (Type C) Endotoxin-free (sterile)</b>	IAX-200-007	100 $\mu$ g   1 mg   3 x 1 mg

## Control ODNs (GpC ODNs)

Inactive control compounds for CpG ODNs do not stimulate TLR9. They are composed of the same sequence as their stimulatory counterparts, but instead of CpG they contain GpC dinucleotides.

PRODUCT NAME	PID	SIZE
<b>ODN 1720 (Control for ODN 1668) Endotoxin-free (sterile)</b>	IAX-200-200	100 $\mu$ g
<b>ODN 2138 (Control for ODN 1826) Endotoxin-free (sterile)</b>	IAX-200-201	100 $\mu$ g
<b>Neutral-ODN (Control for iODNs) Endotoxin-free (sterile)</b>	IAX-200-202	100 $\mu$ g   1 mg   3 x 1 mg
<b>ODN 2118 (Control for ODN 1585) Endotoxin-free (sterile)</b>	IAX-200-203	100 $\mu$ g
<b>ODN M383 (Control for ODN M362) Endotoxin-free (sterile)</b>	IAX-200-204	100 $\mu$ g
<b>ODN 2243 (Control for ODN 2216) Endotoxin-free (sterile)</b>	IAX-200-205	100 $\mu$ g
<b>ODN 2137 (Control for ODN 2006) Endotoxin-free (sterile)</b>	IAX-200-206	100 $\mu$ g

## TLR9 Antagonists — Inhibitory ODNs (iODNs)

In recent years several groups have studied the sequence requirements, specificity, signaling pathways and kinetics of the Toll-like receptor 9 (TLR9) suppression by inhibitory oligonucleotide motifs, which led to a class of novel inhibitory oligonucleotides (iODNs). Subsequently it has been discovered that telomeric DNA repeats (TTAGGG)<sub>n</sub> can block immune activation by CpG-ODNs. Consequently, a classification for iODNs has been proposed.

**Class I:** G-stretch ODNs: TLR9-specific competitors, some iODNs may also affect TLR7 and TLR8 signaling.

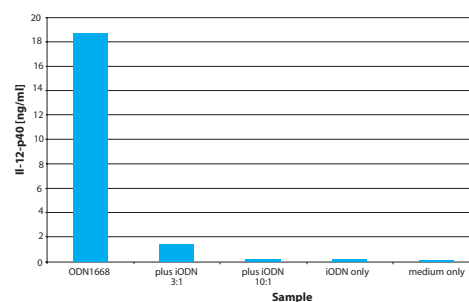
**Class II:** ODNs with telomeric repeats: TLR-independent inhibitors of STAT signaling (cellular uptake via an “ODN receptor”?)

**Class III:** Inhibitors of DNA uptake in a sequence independent manner.

**Class IV:** Long phosphorothioate ODNs as direct competitors of TLR9 signaling in a sequence independent manner.

**LIT:** Immunotherapeutic utility of stimulatory and suppressive oligodeoxynucleotides: K.J. Ishii, et al.; Curr. Opin. Mol. Ther. **6**, 166 (2004) • Inhibitory oligodeoxynucleotides - therapeutic promise for systemic autoimmune diseases?: P. Lenert; Clin. Exp. Immunol. **140**, 1 (2005) • DNA motifs suppressing TLR9 responses: A. Trieu, et al.; Crit. Rev. Immunol. **26**, 527 (2006)

### iODNs — Potent Inhibitors of TLR9 Signaling



**FIGURE 3:** ODN 1668 (Prod. No. IAX-200-001) was added at 50pM/ml to macrophages in a 96-well plate simultaneously together with iODN 2088 (Prod. No. IAX-200-050) at the indicated molar excess, cell supernatants harvested after 24 hours and IL-12-p40 analyzed by cytokine ELISA.

PRODUCT NAME	PID	SIZE
<b>iODN (inhibitory ODN) 2088 Endotoxin-free (sterile)</b>	IAX-200-050	100 µg   1 mg   3 x 1 mg
<b>iODN (inhibitory ODN) (ttaggg)<sub>4</sub> Endotoxin-free (sterile)</b>	IAX-200-051	100 µg   1 mg   3 x 1 mg
<b>G-type iODN (inhibitory ODN) Endotoxin-free (sterile)</b>	IAX-200-052	100 µg   1 mg
<b>Mini-iODN (inhibitory ODN) Endotoxin-free (sterile)</b>	IAX-200-053	100 µg   1 mg
<b>Mega-iODN (inhibitory ODN) Endotoxin-free (sterile)</b>	IAX-200-054	100 µg   1 mg
<b>Duo-iODN (inhibitory ODN) Endotoxin-free (sterile)</b>	IAX-200-055	100 µg   1 mg

► All ODNs include 1 vial of ddWater Endotoxin-free (sterile) (IAX-900-002).

## TECHNICAL NOTE

### Contaminations in TLR Ligands

Specific recognition of different PAMPs by TLRs revealed that only the purest ligands, free of any other immuno-stimulatory contamination, allow to successfully elucidate the role of each TLR. While LPS was thought to not only activate TLR4 but also TLR2, repurification of commercial preparations of both *E. coli* and *Salmonella minnesota* LPS showed that this LPS no longer induces cellular activation through TLR2 [1]. Furthermore it has been shown that purified peptidoglycans activate Nod1 and do not involve TLR2 or TLR4 [2]. Synthetic CpG ODNs (contaminated with LPS) show different activation of certain immune cell subsets.

**LIT :** [1] Repurification of lipopolysaccharide eliminates signalling through both human and murine toll-like receptor 2: M. Hirschfeld, et al.; J. Immunol. **165**, 618 (2000) • [2] Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition: L. H. Travassos, et al.; EMBO Rep. **5**, 1000 (2004)



**offers endotoxin-free and sterile buffers to avoid contaminations upon solubilization of powder form ligands!**

PRODUCT NAME	PID	SIZE
<b>PBS Endotoxin-free (sterile)</b>	IAX-900-001	1.5 ml   10 ml   3 x 10 ml   100 ml
<b>ddWater Endotoxin-free (sterile)</b>	IAX-900-002	1.5 ml   10 ml   3 x 10 ml   100 ml
<b>Physiological Saline [Sodium Chloride 0.9%] Sterile Solution</b>	IAX-900-003	1.5 ml   10 ml   3 x 10 ml   100 ml

## TLR Signaling Antibodies

See [www.adipogen.com](http://www.adipogen.com) for Labeled Antibodies!

ANTIBODIES	PID	SIZE	APPLICATIONS	SPECIES
<a href="#">anti-TLR2, mAb (ABM3A87)</a>	AG-20T-0300	100 µg	FACS, IHC, WB	Hu, Ms
<a href="#">anti-TLR3 (human), mAb (ABM15D5)</a>	AG-20T-0301	100 µg	FACS, WB	Hu
<a href="#">anti-TLR3 (mouse), mAb (ABM24E5)</a>	AG-20T-0302	100 µg	FACS, WB	Ms
<a href="#">anti-TLR4 (human), mAb (ABM19C4)</a>	AG-20T-0303	100 µg	FACS, IHC, WB	Hu
<a href="#">anti-TLR5 (human), mAb (ABM22G1)</a>	AG-20T-0304	100 µg	FACS, IHC	Hu
<a href="#">anti-TLR6 (human), mAb (ABM1B50)</a>	AG-20T-0305	100 µg	FACS, WB	Hu
<a href="#">anti-TLR7 (human), mAb (ABM2C27)</a>	AG-20T-0306	100 µg	FACS, IHC, WB	Hu
<a href="#">anti-TLR8 (human), mAb (ABM15F6)</a>	AG-20T-0307	100 µg	FACS, IHC	Hu
<a href="#">anti-TLR9, mAb (ABM1C51)</a>	AG-20T-0308	100 µg	FACS, IHC, WB	Hu, Ms
<a href="#">anti-TLR9 (mouse), mAb (ABM4D70)</a>	AG-20T-0309	100 µg	FACS, WB	Ms
<a href="#">anti-TLR10 (human), mAb (ABM3C85)</a>	AG-20T-0310	100 µg	WB	Hu

## C-type Lectin Receptors – Dectin-1 & -2

Dectin-1 and Dectin-2 are type II transmembrane C-type lectin pattern recognition receptors that recognize  $\beta$ -glucans and other fungal structures through the carbohydrate-recognition domain (CRD). They function by binding to cell walls of different fungal species triggering intracellular responses like phagocytosis and cytokine production.

PROTEINS	PID
<a href="#">Fc (human):Dectin-1 (mouse) (rec.)</a>	AG-40B-0138
<a href="#">Fc (human):Dectin-2 (mouse) (rec.)</a>	AG-40B-0139

## Non-canonical Inflammasome

The inflammasome is a large cytoplasmic multiprotein complex scaffolded by a pathogen recognition receptor (PRR) that senses infections and endogenous dangers. The inflammasome signals activation of caspase-1, and active caspase-1 then processes IL-1 $\beta$ /IL-18 and triggers a lytic form of programmed necrosis called pyroptosis to mount innate immune responses. In addition Gram-negative bacteria can trigger a caspase-11-dependent (in human caspase-4/5) pathway to induce pyroptosis as well, called the non-canonical inflammasome.

PRODUCT NAME	PID	SIZE	APPLICATIONS	SPECIES
<a href="#">anti-Caspase-4 /11 (p20), mAb (Flamy-1)</a>	AG-20B-0060	100 µg	IP, WB	Hu, Ms
<a href="#">anti-Caspase-11 (mouse), mAb (4E11)</a>	AG-20T-0140	100 µg	FACS, IP, WB	Ms
<a href="#">anti-Caspase-11 (mouse), mAb (8A5)</a>	AG-20T-0139	100 µg	FACS, IP, WB	Ms
<a href="#">anti-Gasdermin D (mouse), pAb (IN110)</a>	AG-25B-0036	100 µg	WB	Ms
<a href="#">Gasdermin D (mouse) ELISA Kit</a>	AG-45B-0011	96 wells	ELISA	Ms

**NEW**

## Gasdermin D Inhibitors

PRODUCT NAME	PID	SIZE
<a href="#">Necrosulfonamide</a>	AG-CR1-3705	5 mg   25 mg
<a href="#">U-73122</a>	AG-CR1-3698	1 mg   5 mg

See our *Inflammasome Brochure!*



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