KEY TLR LIGANDS AND THEIR RECEPTORS

Introduction

In 1996 the Toll protein in *Drosophila* was discovered to be important for defense against microbial infection [1]. One year later a human homolog, now known as Toll-like receptor 4 (TLR4), has been identified through database searches [2]. To date, 13 mouse and 10 human TLRs have been identified. TLRs induce signaling pathways which result in a variety of cellular responses that include the production of interferons (IFNs) and various cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-12. The cytoplasmic region of TLRs consists of a Toll/IL-1 receptor (TIR) domain, showing high similarity to that of the IL-1 receptor family. The extracellular region mainly consists of leucine-rich repeats (LRRs) which are important for the recognition of PAMPs.

TLR Ligands

TLRs are expressed not only on antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages and B cells, but also on specific T cells and non-immune cells (e.g. fibroblasts and epithelial cells). They detect PAMPs of bacteria, viral, fungal and protozoal origin. The various TLRs can be roughly subclassified according to the PAMPs they recognize. TLR1, TLR2 and TLR6 detect lipopeptides, while TLR3, TLR7, TLR8 and TLR9 recognize nucleic acids. TLR5 detects flagellin, while TLR4 recognizes a diverse collection of lipopolysaccharides (LPS). For a comprehensive overview of TLR ligands see pages 2–3. The extracellular region and LRRs either directly or indirectly interact with the ligands. For instance, MD-2, CD14 and LPS-binding protein (LBP) appear to be co-receptors of TLR4, whereas flagellin and unmethylated CpG oligonucleotides appear to interact directly with TLR5 and TLR9, respectively [3-5].

LIT: [1] The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults: B. Lemaitre, et al.; Cell **86,** 973 (1996) ▪ **[2]** A human homologue of the Drosophila Toll protein signals activation of adaptive immunity: R. Medzhitov, et al.; Nature **388,** 394 (1997) ▪ **[3]** LPS, TLR4 and infectious disease diversity: S. I. Miller, et al.; Nat. Rev. Microbiol. **3,** 36 (2005) ▪ **[4]** Direct evidence that toll-like receptor 9 (TLR9) functionally binds plasmid DNA by specific cytosine-phosphate-guanine motif recognition: S. Cornelie, et al.; J. Biol. Chem. **279,** 15124 (2004) ▪ **[5]** Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility: K. D. Smith, et al.; Nat. Immunol. **4,** 1247 (2003)

Toll-like Receptor Signaling

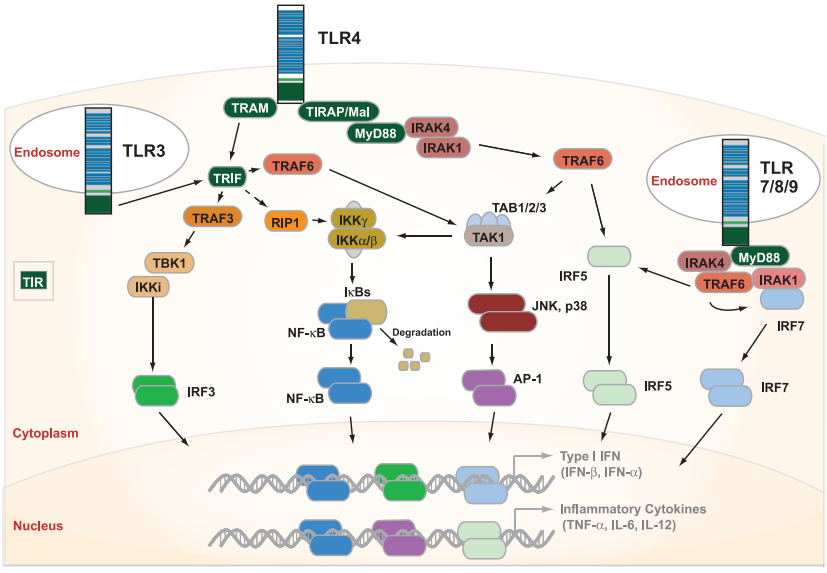


FIGURE 1: Signaling Pathways of TLRs.

TLR ligand binding results in TLR hetero- or homodimerization bringing the cytoplasmic TIR domains to close proximity and subsequently recruit adaptor molecules. Adaptors of TLRs are TIR-domain containing molecules, namely primary MyD88, Mal (TIRAP; TIR-associated protein; MyD88 adaptor-like protein), TRIF (TIR domain-containing adaptor protein-inducing IFN-β; TICAM-1; TIR-containing adaptor molecule-1), TRAM (TRIF-related adaptor molecule; TICAM-2; TIR-containing adaptor molecule-2) and sterile alpha and HEAT-armadillo motifs (SARMs). The induction of different signaling responses by the individual TLRs may be partly explained by the usage of different adaptor molecules.

TLR	Ligands	Origin
TLR1 / TLR2	**TLR1 complexed with TLR2**	
ALX-165-066/-069	Lipoproteins/triacylated lipopeptides: Pam_3CSK_4, JBT3002, OspA	Bacteria, Mycobacteria
	Soluble Lipoproteins	*Neisseria meningitides*
TLR2		
	Bacterial Lipoproteins (BLPs)	Bacteria
	Lipoarabinomannan (LAM)	Mycobacteria
ALX-162-027	MALP-2 (Mycoplasmal Macrophage-activating Lipopeptide-2)	Mycoplasma
	Glycosylphosphatidylinositol (GPI)	*Trypanosoma cruzi*
	Glycolipids	*Treponema maltophilum*
	Porins	*Neisseria sp.*
TLR2/Dectin-1	**TLR2 interacts with Dectin-1**	
	Zymosan	Fungi
TLR3		
	Double-stranded RNA (dsRNA)	Viral
ALX-746-021	Polyinosine-polycytidylic Acid (poly(I:C))	Synthetic
	mRNA	Host
	tRNA	Host/fungi/?
TLR4 / CD14	**TLR4/MD-2 complexed with CD14 and/or LPS-binding Protein [LBP]**	
ALX-581-007 to ALX-581-020 & ALX-581-150	S-Lipopolysaccharides (LPS) (smooth) wild-type (wt) LPS (contains repeated O-polysaccharide units)	Gram-negative bacteria (e.g. *E. coli, Salmonella*)
	R-Lipopolysaccharides (LPS) (rough) mutant (Ra, Rb: extended core-polysaccharide) LPS (S-LPS-like)	Gram-negative bacteria (e.g. *E. coli, Salmonella*)
	R-Lipopolysaccharides (LPS) (rough) mutant (Rc, Rd1, Rd2, Re: short core-polysaccharide) LPS (Lipid A-like)	Gram-negative bacteria (e.g. *E. coli, Salmonella*)
	Flavolipin	*Flavobacterium meningosepticum*
BML-T104	Paclitaxel (Taxol®) (recognizes mouse TLR4)	Plant
	Acyclic Lipid A-like Analog (R-112022)	Synthetic
	Type III Repeat Extra Domain A (EDA)	Host
	LMW Oligosaccharides of Hyaluronic Acid (sHA)	Host
	Polysaccharide Fragments of Heparan Sulfate	Host (only mouse tested)
	Fibrinogen	Host
	Fusion Protein of RSV	Respiratory syncytial virus
	Envelope Proteins of MMTV	Mouse mammary tumor virus
	Glycoinositolphospholipids (GIPLs)	*Trypanosoma cruzi*
	Heat Shock Proteins (HSPs)	TLR2 vs TLR4
TLR4	**TLR4/MD-2 in the absence of CD14 and/or LPS-binding Protein [LBP]**	
see above	R-Lipopolysaccharides (LPS) (rough) mutant (Rc, Rd1, Rd2, Re: short core-polysaccharide) LPS (Lipid A-like)	Gram-negative bacteria (e.g. *E. coli, Salmonella*)
ALX-581-200 to -203	Lipid A (lacks core-polysaccharide: active component of LPS)	Gram-negative bacteria (e.g. *E. coli, Salmonella*)
TLR5		
ALX-522-058	Flagellin	Gram-negative bacteria
TLR6 / TLR2	**TLR6 complexed with TLR2**	
ALX-162-027	MALP-2 (Mycoplasmal Macrophage-activating Lipopeptide-2) / Diacylated Macrophage-activating Lipopeptide-2	Mycoplasma
	Diacylated Lipopeptide FSL-1	Part of the lipoprotein LP44 of *Mycoplasma salivarium*
	Diacylated Lipopeptide Pam_2CSK_4	Synthetic
	Soluble Tuberculosis Factor (STF)	Mycobacteria
TLR7		
ALX-420-039/-040	Imiquimod / Gardiquimod	Synthetic
ALX-420-038	R-848 (Resiquimod)	Synthetic
	S-27610	Synthetic
ALX-480-097	Loxoribine / TOG	Synthetic
	3M-13	Synthetic
	Bropirimine	Synthetic
	Single-stranded RNA (ssRNA) / Polyuridylic acid . K (Poly(U) . K)	Non-viral & viral ssRNA
	U1snRNA	Host
	siRNA	Synthetic
TLR8		
	3M-2	Synthetic
ALX-420-038	R-848 (Resiquimod)	Synthetic
	Single-stranded RNA (ssRNA) / Polyuridylic acid . K (Poly(U) . K)	Non-viral & viral ssRNA
TLR9	**TLR9 (intracellular and extracellular)**	
	Unmethylated CpG DNA	Bacteria / Protozoa/ viral
ALX-746-001 to -006	CpG ODNs	Synthetic
ALX-746-024 to -026	AT-ODNs	Synthetic
	Hemozoin	Protozoa (*Malaria piment*)
TLR10		
	Unknown	
TLR11		
ALX-522-093	Profilin	Protozoa (*Toxoplasma gondii*)
	Uropathogenic *E. coli* (UPECs)	Bacteria

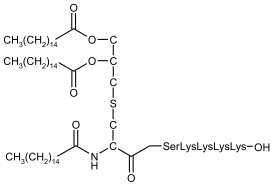
Key Toll-like Receptor Ligands

TLR1/2

Pam$_3$Cys-Ser-(Lys)$_4$. 3HCl

ALX-165-066-M002 2 mg

Selective agonist of TLR1 complexed with TLR2. Cell permeable, water soluble synthetic cationic lipohexapeptide analog of the immunologically active N-terminal portion of bacterial lipoprotein that potently activates monocytes and macrophages.

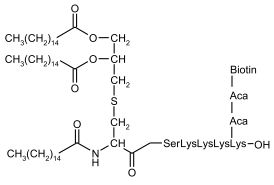


LIT: Lipopeptide derivatives of bacterial lipoprotein constitute potent immune adjuvants combined with or covalently coupled to antigen or hapten: A. Reitermann, et al.; Biol. Chem. Hoppe Seyler **370,** 343 (1989) ▪ **For a comprehensive bibliography please visit our website.**

Pam$_3$Cys-Ser-(Lys)$_4$ (Aca-Aca-Biotin) . 2TFA

ALX-165-069-M001 1 mg

Selective, biotin-labelled ligand of TLR1 complexed with TLR2.



TLR2 & TLR6/2

MALP-2

ALX-162-027-C050 50 µg

ALX-162-034-C500 500 µg

Synthetic. MALP-2 was originally isolated from *Mycoplasma fermentans*. This MALP-2 corresponds to the originally isolated isomer, which expresses potent endotoxin-like activity and approaches in certain experimental systems the toxicity of LPS.

LIT: Purification and partial biochemical characterization of a Mycoplasma fermentans-derived substance that activates macrophages to release nitric oxide, tumor necrosis factor, and interleukin-6: P.F. Muhlradt & M. Frisch; Infect. Immun. **62,** 3801 (1994) ▪ **For a comprehensive bibliography please visit our website.**

TLR3 – Specific Ligand for TLR3

Polyinosinic-polycytidylic acid . K

[poly(I:C) . K (TLRgrade™) (synthetic)]

ALX-746-021-M005 5 mg

Specific ligand for TLR3 [1, 2] and MDA5/Helicard [3].

LIT: [1] The dsRNA binding site of human Toll-like receptor 3: J.K. Bell, et al.; PNAS **103,** 8792 (2006) ▪ **[2]** Subcellular localization of Toll-like receptor 3 in human dendritic cells: M. Matsumoto, et al.; J. Immunol. **171,** 3154 (2003) ▪ **[3]** Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses: H. Kato, et al.; Nature **441,** 101 (2006)

TLR4

Paclitaxel

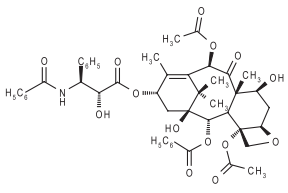
[Taxol®]

BML-T104-0005 5 mg

BML-T104-0025 25 mg

BML-T104-0250 250 mg

Isolated from *Taxus brevifolia*. LPS mimetic in mouse but not human involving the TLR4 signaling pathway.



TLR5

Flagellin (high purity)

ALX-522-058-C010 10 µg

Isolated from *Salmonella typhimurium* strain 14028. **SPECIFICITY:** Binds to human and mouse TLR5. **BIOLOGICAL ACTIVITY:** Activation of TLR5 in human epithelial cell assays based on NF-κB luciferase fusions.

LIT: Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells: F. Sierro, et al.; PNAS **98,** 13722 (2001) ▪ Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation: S.H. Rhee, et al.; PNAS **102,** 13610 (2005)

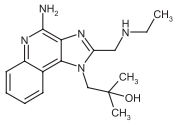
TLR7/8

Gardiquimod

ALX-420-040-M025 25 mg

ALX-420-040-M100 100 mg

Selective ligand for human or mouse TLR7. Induces the activation of NF-κB in HEK 293 cells expressing TLR7. At high concentrations (3µg/ml) slightly activates TLR8. More active than imiquimod (Prod. No. ALX-420-039).



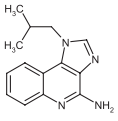
Imiquimod

[R-837]

ALX-420-039-M100 100 mg

ALX-420-039-M250 250 mg

Topical immune response modifier that inhibits angiogenesis. Up-regulates IL-18 and down-regulates MMP-9 through recognition of TLR7 and subsequent activation of MyD88-dependent pathway.



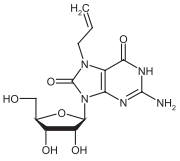
LIT: Imiquimod applied topically: a novel immune response modifier and new class of drug: R.L. Miller, et al.; Int. J. Immunopharmacol. **21,** 1 (1999) ▪ **For a comprehensive bibliography please visit our website.**

Loxoribine

ALX-480-097-M025 25 mg

ALX-480-097-M100 100 mg

Selective ligand for TLR7 via IFN. Activates natural killer cells and primes cytolytic precursor cells for activation by IL-2.



LIT: Loxoribine (7-allyl-8-oxoguanosine) activates natural killer cells and primes cytolytic precursor cells for activation by IL-2: B.L. Pope, et al.; J. Immunol. **151,** 3007 (1993) ▪ **For a comprehensive bibliography please visit our website.**

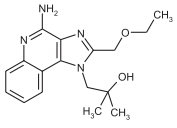
R-848

[S 28463; Resiquimod]

ALX-420-038-M005 5 mg

ALX-420-038-M025 25 mg

Selective ligand for TLR7 in mouse and for TLR7 and TLR8 in human. Potent antitumor and antiviral compound.



LIT: The immune response modifier resiquimod mimics CD40-induced B cell activation: G.A. Bishop, et al.; Cell. Immunol. **208,** 9 (2001) ▪ Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway: H. Hemmi, et al.; Nat. Immunol. **3,** 196 (2002) ▪ **For a comprehensive bibliography please visit our website.**

TLR11

Profilin *(Toxoplasma gondii)* (rec.)

ALX-522-093-C010 10 µg

Produced in *E. coli*. Full length profilin (aa 1-163) from *Toxoplasma gondii* is fused to a N-terminal tag. **SPECIFICITY:** Binds to mouse TLR11.

TLR*grade*™ – LPS & Lipid A Reagents

- Sterile, ready-to-use liquid formulation – No hazardous handling!
- No further purification required!
- Concentration: 1mg/ml
- Ultrapure (≥99.9%)

Product	Prod. No.	Size
LPS from *Salmonella minnesota* R345 (Rb) (TLR*grade*™) (Ready-to-Use)	ALX-581-015-L002	2 ml
LPS from *Salmonella minnesota* R5 (Rc) (TLR*grade*™) (Ready-to-Use)	ALX-581-017-L002	2 ml
LPS from *Salmonella minnesota* R7 (Rd) (TLR*grade*™) (Ready-to-Use)	ALX-581-018-L002	2 ml
LPS from *Salmonella minnesota* R595 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-008-L002	2 ml
LPS from *E. coli*, Serotype EH100 (Ra) (TLR*grade*™) (Ready-to-Use)	ALX-581-010-L002	2 ml
LPS from *E. coli*, Serotype J5 (Rc) (TLR*grade*™) (Ready-to-Use)	ALX-581-014-L002	2 ml
LPS from *E. coli*, Serotype R515 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-007-L002	2 ml
LPS from *E. coli*, Serotype 0111:B4 (TLR*grade*™) (Ready-to-Use)	ALX-581-012-L002	2 ml
LPS from *E. coli*, Serotype 055:B5 (TLR*grade*™) (Ready-to-Use)	ALX-581-013-L002	2 ml
LPS from *Salmonella abortus equi* S-form (TLR*grade*™) (Ready-to-Use)	ALX-581-009-L002	2 ml
LPS from *Salmonella abortus equi* S-form (TLR*grade*™) (Ready-to-Use) (Biotin)	ALX-581-150-R500	500 µl
LPS from *Salmonella typhimurium* S-form (TLR*grade*™) (Ready-to-Use)	ALX-581-011-L002	2 ml
LPS from *Salmonella enteritidis* S-form (TLR*grade*™) (Ready-to-Use)	ALX-581-019-L002	2 ml
LPS from *Salmonella minnesota* S-form (TLR*grade*™) (Ready-to-Use)	ALX-581-020-L002	2 ml
Lipid A from *E. coli*, Serotype R515 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-200-L002	2 ml
Lipid A from *Salmonella minnesota* R595 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-201-L002	2 ml
Monophosphoryl Lipid A [MPL-A] from *E. coli*, Serotype R515 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-203-L001	1 ml
Monophosphoryl Lipid A [MPL-A] from *Salmonella minnesota* R595 (Re) (TLR*grade*™) (Ready-to-Use)	ALX-581-202-L001	1 ml

All the TLR*grade*™ LPS and Lipid A preparations are specific activators of Toll-like receptor (TLR) 4 and **do not activate TLR2 or other TLRs** as determined with splenocytes and macrophages from TLR4 deficient mice (see Figure 1).

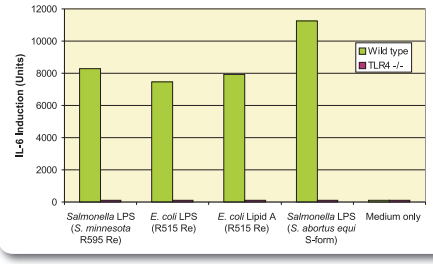


FIGURE 1: Activation of macrophages from TLR4 wild type compared to TLR4 deficient mice by TLR*grade*™ LPS and TLR*grade*™ Lipid A from Enzo Life Sciences. Lipid A or LPS concentrations, which induced maximal activation of TLR4 wild type mouse macrophages, were also applied to TLR4 deficient mouse macrophages (105) as follows: 80ng Salmonella LPS (*S. minnesota* R595 Re), 80ng *E. coli* LPS (R515 Re), 400ng *E. coli* Lipid A (R515 Re) and 400ng Salmonella LPS (*S. abortus* equi S-form). 10 units of IL-6 correspond to the detection limit of the IL-6 ELISA.

Technical Note

Contaminated TLR Ligands

The delicate and 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* showed that LPS no longer induces cellular activation through TLR2 [1-5].** On the other hand highly purified HSP60 [6-7] and HSP70 [8] do not stimulate TLR4 as previously reported [9]. Furthermore it has been shown that purified peptidoglycans activate Nod1 and does not involve TLR2 or TLR4 [10, 11]. Even synthetic CpG ODNs show different activation of certain immune cell subsets when highly purified (TLR*grade*™ CpG ODNs, see next chapter).

LIT: [1] Lipopolysaccharides (LPS) of oral black-pigmented bacteria induce tumor necrosis factor production by LPS-refractory C3H/HeJ macrophages in a way different from that of Salmonella LPS: T. Kirikae, et al.; Infect. Immun. **67,** 1736 (1999) ▪ **[2]** Repurification of lipopolysaccharide eliminates signalling through both human and murine toll-like receptor 2: M. Hirschfeld, et al.; J. Immunol. **165,** 618 (2000) ▪ **[3]** Toll-like receptor **4,** but not toll-like receptor **2,** is a signalling receptor for Escherichia and Salmonella lipopolysaccharides: R.I. Tapping, et al.; J. Immunol. **165,** 5780 (2000) ▪ **[4]** Two lipoproteins extracted from Escherichia coli K-12 LCD25 lipopolysaccharide are the major components responsible for Toll-like receptor 2-mediated signalling: H.K. Lee, et al.; J. Immunol. **168,** 4012 (2002) ▪ **[5]** Murein lipoprotein, peptidoglycan-associated lipoprotein, and outer membrane protein A are present in purified rough and smooth lipopolysaccharides: J. Hellman, et al.; J. Infect. Dis. **188,** 286 (2003) ▪ **[6]** Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor alpha from murine macrophages: B. Gao & M.F. Tsan; J. Biol. Chem. **278,** 22523 (2003) ▪ **[7]** Endotoxin-free heat-shock protein 70 fails to induce APC activation: H. Bausinger, et al.; Eur. J. Immunol. **32,** 3708 (2002) ▪ **[8]** Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages: B. Gao & M.F. Tsan; J. Biol. Chem. **278,** 174 (2003) ▪ **[9]** Interaction of TLR2 and TLR4 ligands with the N-terminal domain of Gp96 amplifies innate and adaptive immune responses: T. Warger, et al.; J. Biol. Chem. **281,** 22545 (2006) ▪ **[10]** Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan: S.E. Girardin, et al.; Science **300,** 1584 (2003) ▪ **[11]** Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition: L. H. Travassos, et al.; EMBO Rep. **5,** 1000 (2004)

Overview on LPS-forms – "R versus S"

Colony morphology is indicative of the O-glycosylation status. Wild-type bacteria form smooth colonies, synthesize "smooth" or S-form LPS that contain O-polysaccharide chains. S-form LPS also contain R-form LPS molecules in variable proportion depending on culture conditions. So-called "rough-mutants" of gram-negative bacteria synthesize "rough" or R-form LPS. These R-forms lack the O-polysaccharide chains and the core saccharide chain may be present in different stages of completion, giving rise to defined R-classes (e.g. Ra, Rb, Rc, Rd, Re). All R-form LPS are devoid of any S-form LPS [1].

The S-form LPS are commonly the preferred choice for whole animal studies, activating TLR4-positive cells strictly dependent on the presence of membrane-anchored or soluble CD14. They also activate the TLR4/MyD88-independent pathway (TRAM/TRIF: type I IFN) and are therefore selective for classical APC expressing CD14 (e.g. monocytes, macrophages, DC) *in vivo*. The *in vivo* activity of S-form LPS may be modulated by increased levels of LBP (LPS-binding protein) during inflammation [2].

The R-form LPS and Lipid A are primarily used in cellular *in vitro* activation studies. They activate TLR4-positive cells independent of the presence of membrane-anchored or soluble CD14. They do not activate the TLR4/MyD88-independent pathway (TRAM/TRIF: type I IFN), but they also activate non-classical APC (PMN/mast cells) *in vivo*. They are very useful for *in vitro* cellular activation assays, where CD14/LBP may be absent or only available in limited amounts. A nontoxic nonpyrogenic derivative of Lipid A is Monophosphoryl Lipid A (MPL-A) exhibiting adjuvant properties that may be used in vaccine development [3].

LIT: [1] R-form LPS, the master key to the activation ofTLR4/MD-2-positive cells: M. Huber, et al.; Eur. J. Immunol. **36,** 701 (2006) ▪ **[2]** CD14 is required for MyD88-independent LPS signaling: Z. Jiang, et al.; Nat. Immunol. **6,** 565 (2005) ▪ **[3]** Role of innate immune factors in the adjuvant activity of monophosphoryl lipid A: M. Martin, et al.; Infect. Immun. **71,** 2498 (2003)

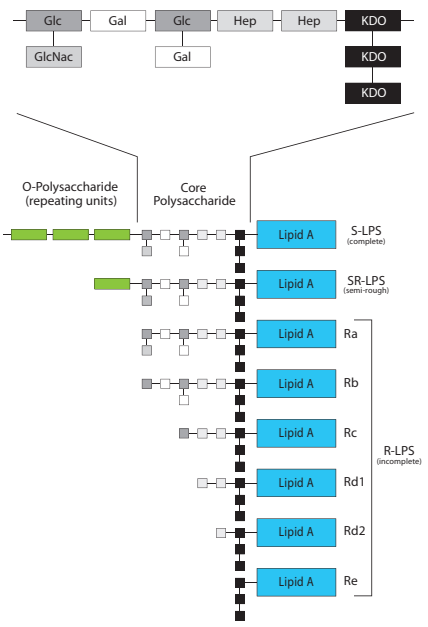


FIGURE 2: Schematic representation of the different LPS chemotypes: GlcNAc = N-Acetylglucosamine; Glc = Glucose; Gal = Galactose; Hep = Heptose; KDO = 2-Keto-3-desoxyoctonate. Adapted from M. Huber, et al.; Eur. J. Immunol. **36,** 701 (2006) [1].

Technical Note

LPS are amphipatic molecules whose hydrophobicity increases with decreasing length of the polysaccharide chain. Therefore Re class LPS and Lipid A are more hydrophobic than Ra class or S-form LPS. The use of Ca^{2+} and Mg^{2+}-free buffers is recommended for the preparation of diluted solutions in order to avoid precipitation and loss of activity for Re-LPS, Lipid A and MLP-A. For *in vivo* applications prepare solutions in glucose instead of PBS.

Salmonella minnesota

Product No.	LPS-form	Constituents	Mac	Dend	B Cells	PMN	Mast
ALX-581-009	S-form	O-polysaccharides-Core-Lipid A	++	++	++	+/-	+/-
ALX-581-011	S-form	O-polysaccharides-Core-Lipid A	++	++	++	+/-	+/-
ALX-581-016	R-form (Ra)	GlcNAc-Glc-Gal-(Glc-Gal)-$(Hep)_{1-2}$-Hep-$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-015	R-form (Rb)	Glc-Gal-(Glc-Gal)-$(Hep)_{1-2}$-Hep-$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-017	R-form (Rc)	Glc-$(Hep)_{1-2}$-Hep-$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-018	R-form (Rd)	Hep-$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-008	R-form (Re)	$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-201	–	Lipid A	++	++	+++	+++	+++
ALX-581-202	–	Monophosphoryl Lipid A (MPL-A)	++	++	+++	+++	+++

E. coli

Product No.	LPS-form	Constituents	Mac	Dend	B Cells	PMN	Mast
ALX-581-012	S-form	O-polysaccharides-Core-Lipid A	++	++	++	+/-	+/-
ALX-581-013	S-form	O-polysaccharides-Core-Lipid A	++	++	++	+/-	+/-
ALX-581-010	R-form (Ra)	Complete E.coli-core (type II)-Lipid A	++	++	+++	+++	+++
ALX-581-014	R-form (Rc)	Glc-$(Hep)_{1-2}$-Hep-$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-007	R-form (Re)	$(KDO)_{2-3}$-Lipid A	++	++	+++	+++	+++
ALX-581-200	–	Lipid A	++	++	+++	+++	+++

CHART LEGEND: Mac = Macrophages, Monocytes ▪ Dend = Dendritic Cells (monocyte-derived) ▪ PMN = Polymorphonuclear Leukocytes (PMN) ▪ B Cells (mouse only) ▪ Mast = Mast Cells ▪ +/- = Weak/Absent activation ▪ ++ = Strong activation ▪ +++ = Very strong activation

CpG ODNs & iODNs – Ligands of TLR9

- **Activity tested**
- **Potent (0.5-5 µg/ml), endotoxin-free, and selective activators for TLR9 as confirmed with TLR9$^{-/-}$ macrophages and splenocytes**
- **Ultrapure**
- **Easy to handle**

Toll-like receptors (TLRs) are widely expressed recognition receptors of the innate immune system. Four out of ten TLRs identified in humans today recognize nucleic acids, which demonstrates the fundamental importance of microbial DNA and RNA in response to pathogenic microorganisms.

Toll-like receptor 9 (TLR9) recognizes unmethylated CpG motifs in viral and bacterial DNA. Signaling through TLR9 can be accomplished with high efficiency with small, synthetic oligodeoxynucleotides (ODNs) containing optimized CpG motifs, which show great promise as potent vaccine adjuvants and inhibitors of Th2-mediated allergic responses.

Yet uncontrolled TLR9 activation can have deleterious consequences, exacerbating inflammatory tissue damage and increasing sensitivity to toxic shock. Inappropriate TLR9-signaling may also be associated with the promotion of autoimmune diseases like B cell hyperreactivity and anti-DNA antibody production in lupus.

Natural and synthetic DNA sequences have been identified that are able to inhibit immune activation through TLR9. These sequences are derived from diverse sources, including viral sequences, mutated CpG sequences, and repeats of the TTAGGG motif present in mammalian telomeres.

Inhibitory oligodeoxynucleotides (iODNs) composed of TTAGGG multimers reproduce this suppressive activity and block the colocalization of CpG DNA with TLR9 within endosomal vesicles. The mechanism of action is not known, but evidence suggests that inhibitory ODNs do not interfere with cellular uptake of CpG ODNs, nor do they simply compete for binding of TLR9. It was shown that iODNs can affect Th1 priming through the inhibition of STAT1, -3, and-4.

Specific inhibitors of TLR9 would represent a valuable tool for understanding TLR9-mediated responses and might serve as potential therapeutics for some autoimmune diseases.

- **Sterile and pyrogen-free water (1 vial) is now included with every order of CpG ODNs, ODN Controls, and iODNs (except for Bulk products)!**
- **Sterile and pyrogen-free PBS is also available (to be ordered separately).**

iODNs – Potent Inhibitors of TLR9 Signaling

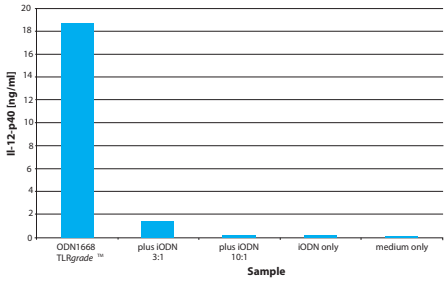


FIGURE 3: TLR*grade*™ CpG ODN 1668 (Prod. No. ALX-746-001) was added at 50pM/ml to macrophages in a 96-well plate simultaneously together with TLR*grade*™ iODN 2088 (Prod. No. ALX-746-250) at the indicated molar excess, cell supernatants harvested after 24 hours and IL-12-p40 analyzed by cytokine ELISA. Similar inhibition rates of >90 (3:1 molar excess) or >95% (10:1) were observed with TLR*grade*™ iODN 2088 and CpG ODN 1668, when analyzed for IL-6.

Selected Literature References

Inhibitory oligonucleotides specifically block effects of stimulatory CpG oligonucleotides in B cells: L.L. Stunz, et al.; Eur. J. Immunol. **32,** 1212 (2002) ▪ Inhibitory oligonucleotides block the induction of AP-1 transcription factor by stimulatory CpG oligonucleotides in B cells: P. Lenert, et al.; Antisense Nuc. Acid Drug Dev. **13,** 143 (2003) ▪ Suppressive oligodeoxynucleotides inhibit Th1 differentiation by blocking IFN-gamma- and IL-12-mediated signaling: H. Shirota, et al.; J. Immunol. **173,** 5002 (2004) ▪ Inhibitors of TLR-9 act on multiple cell subsets in mouse and man in vitro and prevent death in vivo from systemic inflammation: O. Duramad, et al.: J. Immunol. **174,** 5193 (2005) ▪ Suppressive oligodeoxynucleotides protect mice from lethal endotoxic shock: H. Shirota, et al.; J. Immunol. **174,** 4579 (2005) ▪ Therapeutic potential of oligonucleotides expressing immunosuppressive TTAGGG motifs: D.M. Klinman, et al.; Ann. NY Acad. Sci. **1058,** 87 (2005)

Technical Note

Ultrapure TLR*grade*™ ODN preparations are selective activators of TLR9 as confirmed with mouse TLR9$^{-/-}$ macrophages and splenocytes. To guarantee consistent and authentic results, the use of endotoxin-free ddWater or PBS is recommended for solubilization. These products have been subjected to multiple rounds of LPS removal by adsorption with activated charcoal.

LIT: The removal of 14C labeled endotoxin by activated charcoal: A.S. Pegues, et al.; Int. J. Artif. Organs **2,** 153 (1979)

PBS (endotoxin-free)

ALX-505-007-LD15 1.5 ml

Sterile and endotoxin-free PBS for use with TLR*grade*™ or endotoxin-free grade reagents.

ddWater (endotoxin-free)

ALX-505-008-LD15 1.5 ml

Sterile, double distilled and endotoxin-free water for use with TLR*grade*™ or endotoxin-free grade reagents.

Wide Panel of ODNs

Stimulatory ODNs (CpG ODNs)

Three types of stimulatory ODNs have been described, they differ in their sequences and in the type and magnitude of immune responses induced:

Type A are characterized by poly-G motifs with phosphorothioate (PS) linkages at the 5' and 3' ends and a phosphodiester (PO) palindromic CpG-containing sequence in the ODN center. They are very strong inducers of interferon-α (IFN-α) by plasmacytoid dendritic cells (pDC) and especially potent NK cell activators.

Type B are characterized by a full phosphorothioate (PS) backbone with one or more CpG motifs without poly-G motifs. They are weaker inducers of IFN-α, but are potent activators of B cells.

Type C are characterized by a complete phosphorothioate (PS) backbone without poly-G motifs, but also contain palindromic sequences combined with stimulatory CpG motifs. They offer combined features, i.e. induction of INF-α by pDC and activation of B cells.

Optimal sequences in ODNs responsible for activating TLR9 vary among species. Recent studies suggest that the species-selectivity attributed to some CpG motifs compared to GpC motifs in control ODNs may only be observed with phosphorothioate bond linkage (Type B and Type C) rather than ODNs containing „natural" phosphodiester linkages (Type A). The use of TLR*grade*™ reagents shows that relative species selectivity for active CpG ODNs often depends on the concentration used.

LIT: Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities: J. Vollmer, et al.; Eur. J. Immunol. **34,** 251 (2004) ▪ Cutting edge: species-specific TLR9-mediated recognition of CpG and non-CpG phosphorothioate-modified oligonucleotides: T.L. Roberts, et al.; J. Immunol. **174,** 605 (2005)

Control ODNs (GpC ODNs)

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

Inhibitory/Suppressive ODNs (iODNs)

New classes of iODNs defined according to target receptors and activity profile:

- 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 signalling (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)

NEW AT-ODNs!

CpG ODNs are well known for their immunostimulatory effect mediated by the activation of TLR9. It has been shown that not only CpG ODNs but also some AT-rich DNA fragments (AT-ODNs) are able to exert this effect. AT-ODNs with TLR9-activating and immunostimulatory properties were identified in *Lactobacillus gasseri* as well as in the malarial pathogen *Plasmodium falciparum*. AT-ODNs are useful tools for studying the role of TLR9 in the innate immunological response and in inflammation.

AT-ODN-1 (endotoxin-free) (synthetic)

ALX-746-024-C100 100 µg

Synthetic. IFN-γ inducing non-CpG ODN of the AT-type, found in the Malaria genome. TLR9-dependent immune activation. **SEQUENCE:** TATAA*TTTTAATTT*CCAAGA. Nucleotides depicted in italics show the corresponding AT-ODN sequence. Includes 1 vial of ddWater (endotoxin-free) (Prod. No. ALX-505-008).

AT-ODN-2 (endotoxin-free) (synthetic)

ALX-746-025-C100 100 µg

Synthetic. *Lactobacillus gasseri*-derived non-CpG ODN of the AT-type. TLR9-dependent immune activation. **SEQUENCE:** TATAA*TTTTTA*CCAACTAGC. Nucleotides depicted in italics show the corresponding AT-ODN sequence. Includes 1 vial of ddWater (endotoxin-free) (Prod. No. ALX-505-008).

LIT: AT oligonucleotides inducing B lymphocyte activation exist in probiotic Lactobacillus gasseri: H. Kitazawa, et al.; Int. J. Food Microbiol. **65,** 149 (2001) ▪ Augmentation of T(H)-1 type response by immunoactive AT oligonucleotide from lactic acid bacteria via Toll-like receptor 9 signaling: T. Shimosato, et al.; BBRC **326,** 782 (2005) ▪ Strong immunostimulatory activity of AT-oligodeoxynucleotide requires a six-base loop with a self-stabilized 5'-C...G-3' stem structure: T. Shimosato, et al.; Cell. Microbiol. **8,** 485 (2006)

AT-ODN-3 (endotoxin-free) (synthetic)

ALX-746-026-C100 100 µg

Synthetic. IFN-γ inducing non-CpG ODN of the AT-type, found in the Malaria genome. TLR9-dependent immune activation. **SEQUENCE:** TTAACAA*TTTTTA*CCCAAGA. Nucleotides depicted in italics show the corresponding AT-ODN sequence. Includes 1 vial of ddWater (endotoxin-free) (Prod. No. ALX-505-008).

LIT: see Product No. ALX-746-025.

Stimulatory CpG ODNs & Control GpC ODNs

Now available: BULK for in vivo studies!

Product/Sequence	Type	Recommended Species	Prod. No.	Size
ODN 1668 (TLR*grade*™) (synthetic) 5'-tccatgacgttcctgatgct-3'	Type B	Mouse	ALX-746-001-T100	100 tests
ODN 1668 (TLR*grade*™) (synthetic) (BULK) 5'-tccatgacgttcctgatgct-3'	Type B	Mouse	**ALX-746-051-M001**	**1 mg**
ODN 1720 (TLR*grade*™) (synthetic) (Control) 5'-tccatgagcttcctgatgct-3'	Type B	Mouse	ALX-746-200-T100	100 tests
ODN 1826 (TLR*grade*™) (synthetic) 5'-tccatgacgttcctgacgtt-3'	Type B	Mouse	ALX-746-002-T100	100 tests
ODN 1826 (TLR*grade*™) (synthetic) (BULK) 5'-tccatgacgttcctgacgtt-3'	Type B	Mouse	**ALX-746-052-M001**	**1 mg**
ODN 1982 (TLR*grade*™) (synthetic) (Control) 5'-tccatgagcttcctgagctt-3'	Type B	Mouse	ALX-746-201-T100	100 tests
ODN 1585 (TLR*grade*™) (synthetic) 5'-ggGGTCAACGTTGAgggggg-3'	Type A	Mouse	ALX-746-003-T100	100 tests
ODN 2118 (TLR*grade*™) (synthetic) (Control) 5'-ggGGTCAAGCTTGAgggggg-3'	Type A	Mouse	ALX-746-203-T100	100 tests
ODN M362 (TLR*grade*™) (synthetic) 5'-tcgtcgtcgttcgaacgacgttgat-3'	Type C	Human/Mouse	ALX-746-004-T100	100 tests
ODN M383 (TLR*grade*™) (synthetic) (Control) 5'-tgctgctgcttgcaagcagcttgat-3'	Type C	Human/Mouse	ALX-746-204-T100	100 tests
ODN 2216 (TLR*grade*™) (synthetic) 5'-ggGGGACGATCGTCgggggg-3'	Type A	Human	ALX-746-005-T100	100 tests
ODN 2216 (TLR*grade*™) (synthetic) (BULK) 5'-ggGGGACGATCGTCgggggg-3'	Type A	Human	**ALX-746-055-M001**	**1 mg**
ODN 2243 (TLR*grade*™) (synthetic) (Control) 5'-ggGGGAGCATGCTGgggggg-3'	Type A	Human	ALX-746-205-T100	100 tests
ODN 2006 (TLR*grade*™) (synthetic) 5'-tcgtcgttttgtcgttttgtcgtt-3'	Type B	Human/Mouse	ALX-746-006-T100	100 tests
ODN 2006 (TLR*grade*™) (synthetic) (BULK) 5'-tcgtcgttttgtcgttttgtcgtt-3'	Type B	Human/Mouse	**ALX-746-056-M001**	**1 mg**
ODN 2137 (TLR*grade*™) (synthetic) (Control) 5'-tgctgcttttgtgcttttgtgctt-3'	Type B	Human/Mouse	ALX-746-206-T100	100 tests
ODN 2395 (TLR*grade*™) (synthetic) 5'-tcgtcgttttcggcgcgcgccg-3'	Type C	Human/Mouse	ALX-746-020-T100	100 tests

NOTE: Lower case letters indicate phosphorothioate linkage.

Inhibitory ODNs (iODNs)

Product/Sequence	Prod. No.	Size
iODN 2088 (class I) (endotoxin-free) (synthetic) 5'-tcctggcggggaagt-3'	ALX-746-250-T050	50 tests
iODN 2088 (class I) (endotoxin-free) (synthetic) (BULK) 5'-tcctggcggggaagt-3'	**ALX-746-350-M001**	**1 mg**
iODN (ttaggg)$_4$ (class II) (endotoxin-free) (synthetic) 5'-tttagggttagggttagggttaggg-3'	ALX-746-251-T050	50 tests
iODN (ttaggg)$_4$ (class II) (endotoxin-free) (synthetic) (BULK) 5'-tttagggttagggttagggttaggg-3'	**ALX-746-351-M001**	**1 mg**
***G-type*-iODN (class I) (endotoxin-free) (synthetic)** 5'-ctcctattggggtttcctat-3'	ALX-746-252-C100	100 µg
***G-type*-iODN (class I) (endotoxin-free) (synthetic) (BULK)** 5'-ctcctattggggtttcctat-3'	**ALX-746-352-M001**	**1 mg**
***Core*-iODN (class I) (endotoxin-free) (synthetic)** 5'-tcctggagggg-3'	ALX-746-253-C100	100 µg
***Core*-iODN (class I) (endotoxin-free) (synthetic) (BULK)** 5'-tcctggagggg-3'	**ALX-746-353-M001**	**1 mg**
***Super*-iODN (class I/II hybrid) (endotoxin-free) (synthetic)** 5'-cctcaatagggtgagggg-3'	ALX-746-254-C100	100 µg
***Super*-iODN (class I/II hybrid) (endotoxin-free) (synthetic) (BULK)** 5'-cctcaatagggtgagggg-3'	**ALX-746-354-M001**	**1 mg**
***Dual*-iODN (class I) (endotoxin-free) (synthetic)** 5'-tgctcctggaggggttgt-3'	ALX-746-255-C100	100 µg
***Dual*-iODN (class I) (endotoxin-free) (synthetic) (BULK)** 5'-tgctcctggaggggttgt-3'	**ALX-746-355-M001**	**1 mg**

Control Compounds

***Neutral*-ODN (Control for iODNs) (endotoxin-free) (synthetic)** 5'tcctgcaggttaagt-3'	ALX-746-256-C100	100 µg
***Neutral*-ODN (Control for iODNs) (endotoxin-free) (synthetic) (BULK)** 5'-tcctgcaggttaagt-3'	**ALX-746-356-M001**	**1 mg**

iODN	Use
iODN 2088	Potently inhibits TLR9, but may also affect TLR7 and TLR8 signaling.
iODN (ttaggg)$_4$	Potently inhibits STAT signaling, independent of TLR signaling.
***G-type*-iODN**	Potently inhibits TLR9, but without additional effect on TLR7 and TLR8 signaling
***Core*-iODN**	Mini-iODN, which potently inhibits TLR9, but may also affect TLR7 and TLR8 signaling.
***Super*-iODN**	Potently inhibits TLRs and STAT signaling.
***Dual*-iODN**	Potently inhibits TLR9 and TLR7 signaling.
***Neutral*-ODN**	Inactive (neutral) control ODN without agonistic or antagonistic activity.

TABLE: iODNs and their activities.

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

Antibodies to Toll-like Receptors

Product/Clone	Source/Isotype	Species	Application	Prod. No.	Format	Size
TLR1 [Toll-like Receptor 1; CD281]						
TLR1 (human), mAb Clone: GD2.F4	Mouse IgG1	Human	FC	ALX-804-130-C100 ALX-804-130PF-C100	 PF	100 µg 100 µg
TLR1, pAb	From rabbit	Human, mouse, rat	FC, WB	ALX-215-011-C100		100 µg
TLR2 [Toll-like Receptor 2; CD282]						
TLR2 (human), mAb Clone: TL2.1	Mouse IgG2a	Human	ELISA, FC, IHC, IP	ALX-804-323-C050 ALX-804-323PF-C050 ALX-804-323PF-C100 ALX-804-323F-T100	 PF PF FITC	50 µg 50 µg 100 µg 100 tests
TLR2 (human), mAb Clone: TL2.3	Mouse IgG2a	Human	ELISA, IHC (FS), ICC	ALX-804-324-C050 ALX-804-324PF-C100	 PF	50 µg 100 µg
TLR2 (human), pAb	From goat	Human	FC, IHC (PS), ICC, WB	ALX-210-852-C200		200 µg
TLR2, pAb	From rabbit	Human, mouse	WB	ALX-210-369-C100		100 µg
TLR3 [Toll-like Receptor 3; CD283]						
TLR3, mAb Clone: 40C1285.6	Mouse IgG1	Human, canine	FC, IHC (PS), ICC, IP, WB	ALX-804-362-C100 ALX-804-362B-C100 ALX-804-362F-C100 ALX-804-362R-C100	 Biotin FITC R-PE	100 µg 100 µg 100 µg 100 µg
TLR3 (human), mAb Clone: TLR3.7	Mouse IgG1	Human	FC, ICC, IP	ALX-804-474-C050		50 µg
TLR3 (mouse), pAb	From rabbit	Mouse	FC, IHC (FS, PS), WB	ALX-210-367-R200		200 µl
TLR4 [Toll-like Receptor 4; CD284] and TLR4/MD-2 Complex						
TLR4 (human), mAb Clone: HTA125	Mouse IgG2a	Human	FC, IP, FUNC (Blocks activation of monocytes with LPS)	ALX-804-419-C100 ALX-804-419PF-C100 ALX-804-419F-T100	 PF FITC	100 µg 100 µg 100 tests
TLR4, pAb	From goat	Human, mouse	IHC (PS), ICC, WB	ALX-210-638-C200		200 µg
TLR4/MD-2 (mouse), mAb Clone: MTS510	Rat IgG2a	Mouse	FC, IP, FUNC (Inhibition of LPS-induced cytokine production)	ALX-804-430-C100 ALX-804-430PF-C100 ALX-804-430F-T100	 PF FITC	100 µg 100 µg 100 tests
TLR5 [Toll-like Receptor 5; CD285]						
TLR5, mAb Clone: 19D759.2	Mouse IgG2a	Human, mouse, canine	FC, IHC (FS), WB	ALX-804-598-C100 ALX-804-598F-C100 ALX-804-598R-C100	 FITC R-PE	100 µg 100 µg 100 µg
TLR5, mAb Clone: 85B152.5	Mouse IgG2a	Human, mouse, canine	FC, WB	ALX-804-597-C100 ALX-804-597F-C100 ALX-804-597R-C100	 FITC R-PE	100 µg 100 µg 100 µg
TLR5 (human), pAb	From goat	Human	FC, IHC (PS), ICC, WB	ALX-210-853-C200		200 µg

Antibodies to MD-2

NEW

MD-2 (human), mAb (10A8)

ALX-803-324-C100 100 µg

CLONE: 10A8. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human MD-2. **SPECIFICITY:** Recognizes all forms of soluble human MD-2, but not membrane bound MD-2. Does not cross-react with mouse MD-2. **APPLICATION:** ELISA, WB.

LIT: Characterization of monoclonal antibodies to human soluble MD-2 protein: S. Viriyakosol, et al.; Hybridoma **25,** 349 (2006)

MD-2 (human), mAb (4H1)

ALX-803-325-C100 100 µg

CLONE: 4H1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human MD-2. **SPECIFICITY:** Recognizes all forms of soluble human MD-2, but not membrane bound MD-2. Does not cross-react with mouse MD-2. **APPLICATION:** ELISA, WB.

LIT: Characterization of monoclonal antibodies to human soluble MD-2 protein: S. Viriyakosol, et al.; Hybridoma **25,** 349 (2006)

MD-2, pAb

ALX-215-063-C100 100 µg

From rabbit. **IMMUNOGEN:** Full length recombinant human MD-2. **SPECIFICITY:** Recognizes human, mouse and rat MD-2. Detects a band of ~30kDa by Western blot. **APPLICATION:** IHC (PS), WB.

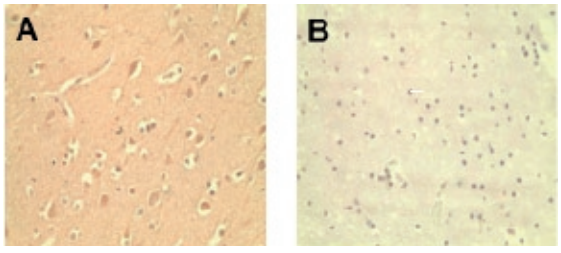


FIGURE: Immunohistochemical staining of normal human brain using MD-2, pAb (Prod. No. ALX-215-063) at 10µg/ml (A) or using a control rabbit Ig at 10µg/ml (B).

Product/Clone	Source/Isotype	Species	Application	Prod. No.	Format	Size
TLR6 [Toll-like Receptor 6; CD286]						
TLR6 (human), mAb Clone: 86B1153.2	Mouse IgG1	Human	FC, IHC (PS)	ALX-804-363-C100 ALX-804-363B-C100 ALX-804-363F-C100	 Biotin FITC	100 µg 100 µg 100 µg
TLR6, pAb	From rabbit	Human, mouse	FC, WB	ALX-210-873-C100		100 µg
TLR7 [Toll-like Receptor 7]						
TLR7 (human), mAb Clone: 4F4	Mouse IgG1	Human	ELISA, WB	ALX-804-654-R100		100 µl
TLR7, pAb	From rabbit	Human, mouse, rat	FC, ICC, IHC (FS, PS), IP, WB	ALX-210-874-C100 ALX-210-874PF-C100 ALX-210-874F-C100	 PF FITC	100 µg 100 µg 100 µg
TLR8 [Toll-like Receptor 8; CD288]						
TLR8, mAb Clone: 44C143	Mouse IgG1	Human, mouse	FC, IHC (PS), WB	ALX-804-376-C100 ALX-804-376B-C100 ALX-804-376F-C100 ALX-804-376R-C100	 Biotin FITC R-PE	100 µg 100 µg 100 µg 100 µg
TLR8, pAb	From goat	Human, mouse	IHC (PS), ICC, WB	ALX-210-639-C200		200 µg
TLR9 [Toll-like Receptor 9; CD289]						
TLR9, mAb Clone: 26C593.2	Mouse IgG1	Human, mouse, rat, canine	FC, ICC, IHC (FS, PS), WB	ALX-804-364-C100 ALX-804-364B-C100 ALX-804-364F-C100 ALX-804-364R-C100	 Biotin FITC R-PE	100 µg 100 µg 100 µg 100 µg
TLR9 (human), pAb	From goat	Human, mouse	ICC, IHC (PS), WB	ALX-210-642-C200		200 µg
TLR9 (mouse), pAb	From goat	Human, mouse	IHC (PS), ICC, WB	ALX-210-637-C200		200 µg
TLR9, pAb	From rabbit	Human, mouse	FC, ICC, IHC (FS, PS), WB	ALX-210-368-C100		100 µg
TLR10 [Toll-like Receptor 10; CD290]						
TLR10 (human), mAb Clone: 158C1114	Mouse IgG1	Human	FC, WB	ALX-804-407-C100		100 µg
TLR10 (human), pAb	From goat	Human	ICC, IHC (PS), WB	ALX-210-643-C200		200 µg
TLR10 (mouse/rat), pAb	From rabbit	Mouse, rat	ELISA, WB	ALX-215-014-C100		100 µg
TLR11 [Toll-like Receptor 11]						
TLR11, pAb	From rabbit	Mouse, rat	WB	ALX-215-013-C100		100 µg
TLR12 [Toll-like Receptor 12]						
TLR12 (mouse), mAb Clone: 15F1215	Mouse IgM	Mouse	WB	ALX-804-596-R200		200 µl
TLR12, pAb	From rabbit	Mouse, rat	FC, WB	ALX-215-012-C100		100 µg

Toll-like Receptor Proteins

TLR1 (human):Fc (human) (rec.)	Produced in mouse NSO/1 cells. The extracellular domain of human TLR1 is fused to the Fc portion of human IgG1.	ALX-522-050-C050	50 µg
TLR4 (mouse):Fc (human) (rec.) (aa 24-334)	Produced in HEK 293 cells. The N-terminal region of the extracellular domain of mouse TLR4 (aa 24-334) is fused at the C-terminus to the Fc portion of human IgG1 and a linker peptide (2 aa).	ALX-522-073-C050	50 µg
TLR6 (mouse):Fc (human) (rec.) (aa 24-594)	Produced in HEK 293 cells. The extracellular domain of mouse TLR6 (aa 24-594) is fused at the C-terminus to the Fc portion of human IgG1 and a linker peptide (10 aa).	ALX-522-075-C050	50 µg

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

NEW Endogenous Danger Signal Molecules for TLR4!

Hyaluronic Acid Fragments (<1,500 Da) (endotoxin-free) (Ready-to-Use)

[sHA; Hyaluronan Oligosaccharide]

ALX-580-004-C250 250 µg

Isolated from bacteria. **PURITY:** ≥ 95% (HPLC); Absence of detectable protein or DNA contaminants with agonistic TLR activity. Endotoxin tested. The purity and size range of HA oligomer mixture has been confirmed by HPLC analysis and mass spectrometry. **ENDOTOXIN CONTENT:** <0.02EU/µg. **QUANTITY:** Sufficient for ~100 tests in 100µl assays.

LIT: Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4: C. Termeer, et al.; J. Exp. Med. **195,** 99 (2002)

Hyaluronic Acid Fragments (1,500-3,000 Da) (endotoxin-free) (Ready-to-Use)

[sHA; Hyaluronan Oligosaccharide]

ALX-580-005-C250 250 µg

Isolated from bacteria. **PURITY:** ≥ 95% (HPLC); Absence of detectable protein or DNA contaminants with agonistic TLR activity. Endotoxin tested. The purity and size range of HA oligomer mixture has been confirmed by HPLC analysis and mass spectrometry. **ENDOTOXIN CONTENT:** <0.02EU/µg. **QUANTITY:** Sufficient for ~100 tests in 100µl assays.

LIT: Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4: C. Termeer, et al.; J. Exp. Med. **195,** 99 (2002)

NEW Ligands for Intracellular DNA Sensors!

Poly(deoxyadenosine:deoxythymidine) (endotoxin-free) (synthetic)

[Poly(dA:dT)]

ALX-746-022-C050 50 µg

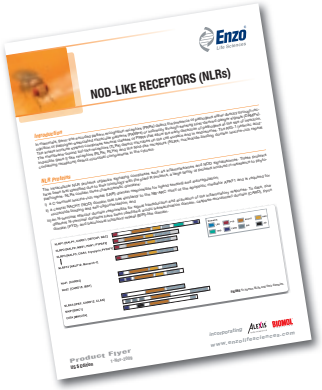
Synthetic. Strong IFN inducer, **independent** of IRF3. AIM2 inflammasome activator.

Oligo(deoxyadenosine:deoxythymidine) (endotoxin-free) (synthetic)

[Oligo(dA:dT)]

ALX-746-023-C050 50 µg

Synthetic. Strong IFN inducer, **dependent** of IRF3.



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