

InvivoGen Insight

An Insightful Look At InvivoGen's Innovative Products

Since the discovery of the Toll-like receptors less than a decade ago, our understanding on how these receptors detect pathogen invasion and induce an immune response has tremendously progressed. Still, a lot remains to be unraveled. As a leader in the TLR field, InvivoGen strives to continuously provide new TLR products.

In this Insight issue, you will discover that our collection of 293/TLR clones has widened, as well as our list of TLR ligands. We have also added to our TLR product line inhibitors of proteins involved in TLR

signaling, such as PKC or MAP kinases. Furthermore, we are introducing a new group of ready-made psiRNA plasmids expressing short hairpin RNAs that target the TLR genes. These shRNAs, which silence TLR genes by at least 70%, will be useful in studies aimed at deciphering the TLR signaling pathway.

Lastly, InvivoGen provides a large collection of genes that present an interest in human therapy. These genes are available as full length open reading frames in a plasmid called pORF.

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TLR9 Recognition and Signaling: An Update

Toll-like receptor 9 (TLR9) recognizes unmethylated bacterial CpG DNA and initiates a signaling cascade leading to the production of proinflammatory cytokines. The stimulatory effect of CpG DNA is conferred by unmethylated CpG dinucleotides in particular base contexts (CpG motifs) that also determine the species-specific activity of CpG DNA. CpG motifs containing the core sequence GACGTT highly stimulate mouse TLR9, whereas CpG motifs containing more than one CpG and the core sequence GTCGTT are optimal inducers of human TLR9.

Accumulating evidence suggests that CpG DNA and TLR9 interact in intracellular compartments. For example, lipofection increases the stimulatory activity of CpG DNA and chloroquine, an inhibitor of endosomal acidification, prevents TLR9 signaling. Recent studies show that TLR9 is expressed in the ER of resting cells in contrast to most TLRs that are located on the plasma membrane¹. As CpG DNA is internalized through endocytosis, TLR9 relocates to the entry site of CpG DNA. The accumulation of CpG DNA and TLR9 in the endosomes leads to their co-localization within the same vesicles, and induces the recruitment of MyD88 to initiate signaling².

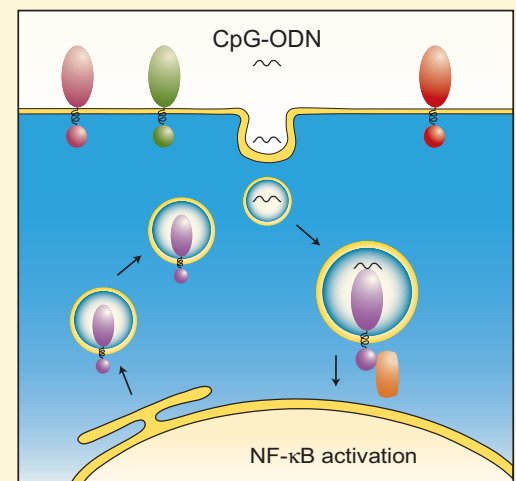
CpG DNA binds directly to TLR9. A potential CpG DNA binding domain was identified within TLR9 that shares homology with the methyl-CpG-DNA binding domain (MBD) of MBD proteins, a family of proteins implicated in gene silencing and chromatin remodeling³.

TLR9 recognizes specifically CpG DNA that is unmethylated and single stranded (ss). Methylation of the cytosine within the CpG motif strongly reduces the affinity of TLR9^{3,4}. In addition, double stranded (ds) CpG DNA is a weak stimulator of TLR9 compared to its ss counterpart³. This observation seems to contradict the findings that genomic *E. coli* DNA activates TLR9. We have found that *E. coli* DNA induces a poor response in TLR9-transfected HEK293 cells. In contrast, we observed that short ss fragments of *E. coli* DNA, generated by sonication and denaturation, were able to activate TLR9. A possible explanation is that upon endocytosis, ds CpG DNA is degraded into small ss CpG motifs that can activate TLR9. CpG DNA containing a phosphodiester (PD) backbone interact with TLR9 in a CpG sequence specific manner. In contrast, phosphorothioate (PTO)-protected ODNs bind to

TLR9 in a CpG-independent manner^{2,3}, but show a CpG-dependent stimulatory activity³. This difference between PD and PTO backbones suggests that the structure of the ODN influences the binding to TLR9 and the subsequent cellular activation.

Three types of CpG ODNs have been identified based on their distinct activity on plasmacytoid dendritic cells: type A (or D), B (or K) and C. Unlike CpG ODN-B, CpG ODN-A induces only modest NF- κ B activation in B cells or HEK293 cells expressing TLR9, suggesting that a distinct cofactor may be required for activation^{2,5}. Clearly, there is still much to learn before we fully understand CpG DNA-TLR9 interactions. This understanding is the key to design novel therapeutic agents for the treatment of autoimmune diseases or cancers.

1. Latz E. *et al.*, 2004. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol.* 5(2):190-8.
2. Takeshita F. *et al.*, 2004. Signal transduction pathways mediated by the interaction of CpG DNA with Toll-like receptor 9. *Semin Immunol.* 16(1):17-22.
3. Rutz M. *et al.*, 2004. Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. *Eur J Immunol.* 34(9):2541-50.
4. Corneliu S. *et al.*, 2004. Direct evidence that toll-like receptor 9 (TLR9) functionally binds plasmid DNA by specific cytosine-phosphate-guanine motif recognition. *J Biol Chem.* 279(15):15124-9.
5. Verthelyi D, Zeuner RA., 2003. Differential signaling by CpG DNA in DCs and B cells: not just TLR9. *Trends Immunol.* 24(10):519-22.



293/TLR Clones

Cells that constitutively express a given TLR gene at high levels are valuable tools for many applications, such as the study of the mechanisms involved in TLR recognition or signaling, and the development of new potential therapeutic drugs. In addition to 293/hTLR clones that express human TLR genes, InvivoGen provides two new families of 293/TLR clones: 293/mTLR clones and 293/hTLR-HA clones.

293/mTLR Clones

Mouse TLR Expressing Cells

293/mTLR clones are HEK293 cells stably transfected with a pUNO-TLR or pDUO-TLR plasmid expressing either one or two mouse TLR genes. Expression of the mouse TLR genes has been verified by RT-PCR and their functionality confirmed by performing transient transfection assays with an NF- κ B inducible reporter plasmid.

293/mTLR clones are grown in standard DMEM medium with 10% FBS supplemented with blasticidin (10 μ g/ml). Cells are provided frozen in a cryotube containing 3-5 x 10⁶ cells and supplied with 100 μ l of blasticidin at 10 mg/ml. Cells are shipped on dry ice.

Product	Catalog Code	Price
293/mTLR1	293-mtlr1	\$700
293/mTLR1/2	293-mtlr1/2	\$800
293/mTLR2	293-mtlr2	\$700
293/mTLR2/6	293-mtlr2/6	\$800
293/mTLR3	293-mtlr3	\$700
293/mTLR4	293-mtlr4	\$700
293/mTLR5	293-mtlr5	\$700
293/mTLR6	293-mtlr6	\$700
293/mTLR7	293-mtlr7	\$700
293/mTLR9	293-mtlr9	\$700

293/hTLR-HA Clones

HA-tagged TLR Expressing Cells

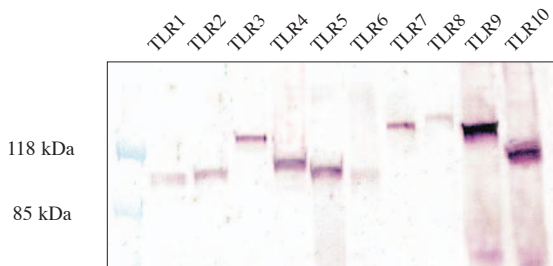
293/hTLR-HA clones were obtained by stably transfecting HEK293 cells with a pUNO-TLR-HA plasmid. pUNO-TLR-HA plasmids express TLR genes that have been fused at the 3' end to the influenza hemagglutinine (HA) tag. This tag is the epitope of a very efficient and specific monoclonal antibody. The use of human HA-tagged TLR genes provides a simple and convenient method to detect the expression of the TLR genes by Western blot. All human TLR genes can be detected using the same primary antibody. Expression of the human HA-tagged TLR genes has been verified by RT-PCR and Western blot analysis. Their functionality has been tested by transient transfection using an NF- κ B inducible reporter plasmid.

293/hTLR-HA clones are grown in standard DMEM medium with 10% FBS supplemented with blasticidin (10 μ g/ml). Each vial of 293/hTLR-HA clone contains 3-5 x 10⁶ cells and is supplied with 100 μ l of blasticidin at 10 mg/ml. Cells are shipped on dry ice.

Product	Catalog Code	Price
293/hTLR1-HA	293-htlr1ha	\$800
293/hTLR2-HA	293-htlr2ha	\$800
293/hTLR3-HA	293-htlr3ha	\$800
293/hTLR4-HA	293-htlr4ha	\$800
293/hTLR5-HA	293-htlr5ha	\$800
293/hTLR6-HA	293-htlr6ha	\$800
293/hTLR7-HA	293-htlr7ha	\$800
293/hTLR8-HA	293-htlr8ha	\$800
293/hTLR9-HA	293-htlr9ha	\$800
293/hTLR10-HA	293-htlr10ha	\$800

Related products

Blasticidin (100 mg)	ant-bl-1	\$180
Anti-HA tag (250 μ l)	ab-hatag	\$175



TLR Ligands	TLR2	TLR3	TLR4*	TLR5	TLR7	TLR8	TLR9
HKLM	+++	-	-	-	-	-	-
Poly(I:C)	+	++++	++	++	+	+	+
LPS	-	-	++++	-	-	-	-
Flagellin	-	-	++	++++	-	-	-
R848	-	-	-	-	+++	+++	-
ssPolyU	-	-	-	-	-	+	-
CpG-ODN-B	-	-	-	-	-	-	++

293/hTLR-HA clones were transiently transfected with an NF- κ B inducible reporter plasmid (and cotransfected with a MD2-CD14 expressing plasmid in the case of TLR4). Forty-eight hours later, cells were stimulated with various ligands. Induction levels were monitored 12 to 24 hours later.

HKLM: Heat-killed *Lysteria monocytogenes*, LPS: *E. coli* lipopolysaccharide, ssPolyU: single-stranded polyuridine complexed with LyoVec™

TLR Ligand Screening Service



InvivoGen has developed a high-throughput technology to evaluate TLR ligands. Based on this technology, InvivoGen is now offering a TLR ligand screening service. This service provides a rapid, reliable and cost-effective way to screen for new molecules that have the ability to stimulate the TLRs. Send us the molecules you believe are potential TLR ligands, and in less than 2 weeks we will provide you with a report detailing which products activate the TLRs and the specific TLR(s) involved.

Key Service Features

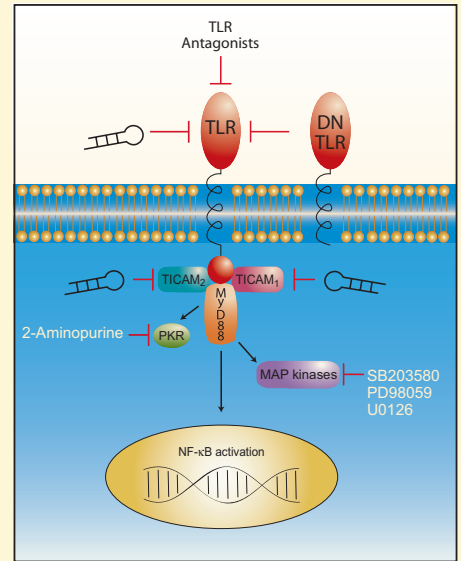
- Technological expertise
- Qualitative and quantitative analysis
- Short turnaround time
- Cost effective

Inquire for pricing or check our website for more information

Deciphering TLR Signaling

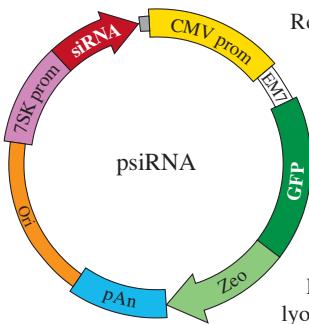
Toll-like receptors share common structures and signaling that leads to NF- κ B activation. However, recent data suggest that NF- κ B activation may not be uniform and that distinct TLR-ligand associations may trigger multiple pathways. InvivoGen provides a number of tools that should prove useful to help elucidate the molecular mechanisms involved in TLR signaling.

- **Dominant Negative TLRs** - InvivoGen has engineered TLR genes deleted for the TIR domain (TLR- Δ TIR) that act as dominant negatives. Human and mouse TLR- Δ TIR genes are available in an expression vector called pZERO-TLR. For more information, check our website.
- **shRNAs Targeting TLR and Adaptor Genes** - Using the siRNA Wizard algorithm, InvivoGen has designed and cloned sequences that code for short hairpin RNAs (shRNAs) that functionally silence a number of TLR and adaptor genes. These shRNAs are available as ready-made psiRNA plasmids.
- **Inhibitors of TLR Signaling** - This product line includes compounds that antagonize TLR binding (polymixin B), prevent signaling of TLRs within the endosome (chloroquine), or inhibit kinases involved in intracellular signaling (see below).



Ready-Made psiRNA

Silence Toll-like Receptor and Adaptor Genes



Ready-made psiRNA is a new family of plasmids expressing functional siRNAs. They encode shRNAs that target key genes, such as TLR and adaptor genes, and induce their silencing by at least 70% through RNAi.

Ready-made psiRNA plasmids feature a GFP::Zeo fusion gene that allows simple monitoring of transfection efficiency and selection in both *E. coli* and mammalian cells.

Each ready-made psiRNA is provided as 20 μ g lyophilized DNA. **Price \$395**

Target Gene	Catalog Code (human)	Catalog Code (mouse)
TLR1	psiRNA3gz21-htrl1	psiRNA3gz21-mtrl1
TLR2	psiRNA3gz21-htrl2	psiRNA3gz21-mtrl2
TLR3	psiRNA3gz21-htrl3	
TLR4	psiRNA3gz21-htrl4	psiRNA3gz21-mtrl4
TLR5	psiRNA3gz21-htrl5	psiRNA3gz21-mtrl5
TLR6	psiRNA3gz21-htrl6	psiRNA3gz21-mtrl6
TLR7	psiRNA3gz21-htrl7	psiRNA3gz21-mtrl7
TLR8	psiRNA3gz21-htrl8	
TLR9	psiRNA3gz21-htrl9	psiRNA3gz21-mtrl9
MyD88	psiRNA3gz21-hmyd88	
TICAM1 (TRIF)	psiRNA3gz21-hticam1	psiRNA3gz21-mticam1
TICAM2 (TRAM)	psiRNA3gz21-hticam2	psiRNA3gz21-mticam2
TIRAP	psiRNA3gz21-htirap	

For an updated list, go to

http://www.invivogen.com/siRNA/siRNA_ready.htm

TLR Ligands

FSL-1 - TLR2/TLR6 ligand

* MALP2 Replacement *

FSL-1 is a lipoprotein synthesized on the basis of the NH2 terminus of a *Mycoplasma salivarium* lipoprotein. Similarly to MALP2, FSL-1 contains a diacylated cysteine residue and acts as a potent activator of NF- κ B upon recognition by TLR2 and TLR6. FSL-1 is more favorable than MALP2 as it exhibits a higher stimulatory activity than MALP2 and is water soluble.

Working concentration: 10 ng-1 μ g/ml #trl-fsl (100 μ g) \$250

Heat-killed *Legionella pneumophila* (HKLP) - Multiple TLR ligand

Heat-killed *Legionella pneumophila*, a gram negative bacteria responsible for legionnaires' disease, induces a strong inflammatory response. This response seems to be triggered by different TLRs, including TLR2 and TLR4.

Working concentration: 10⁸ cells/ml #trl-hklp (10⁸ cells) \$120

Sheared *E. coli* ssDNA - TLR9 ligand

Sheared *E. coli* ssDNA are short single-stranded DNA fragments produced by treating genomic *E. coli* DNA with ultrasound followed by heat denaturation. They are complexed with LyoVec™, a lipid-based transfection reagent, to allow penetration of the DNA in the cells. Sheared *E. coli* ssDNA activate TLR9 as efficiently as CpG-ODNs and in a species-independent manner.

Working concentration: 10 μ g/ml #trl-ssec (200 μ g) \$140

TLR Signaling Inhibitors

2-Aminopurine - PKR Inhibitor

Working concentration: 1-10 mM #trl-apr (250 mg) \$80

LY294002 - PI3K Inhibitor (TLR9 signaling)

Working concentration: 50-100 μ M #trl-ly29 (5 mg) \$70

PD98059 - MAP Kinase Kinase Inhibitor

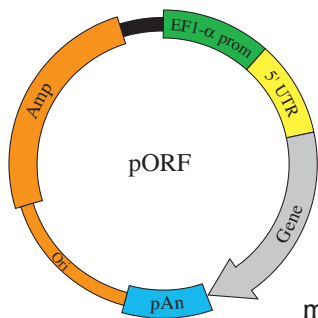
Working concentration: 50-100 μ M #trl-pd98 (10 mg) \$70

SB203580 - p38/RK MAP Kinase Inhibitor

Working concentration: 1-20 μ M #trl-sb20 (5 mg) \$80

U0126 - MEK1 and MEK2 Inhibitor

Working concentration: 10-50 μ M #trl-u0126 (5 mg) \$80



pORF

The Largest Collection of Immune Genes

pORF is a family of plasmids expressing a large choice of human and mouse genes, including genes involved in the immune response. pORF plasmids are designed to provide the scientific community with a reliable and updated list of genes that can be directly transfected into mammalian cells or can be easily subcloned into another vector of choice.

Full-length Open Reading Frames (ORFs)

Each gene is cloned from the ATG to Stop codon. Genes encoding proteins that are naturally secreted include their native signal sequence which is generally located at the 5' end of the coding sequence. Each gene is fully sequenced. The sequence is available online at www.invivogen.com or can be emailed.

Suitable for Expression in Mammalian Cells

Each ORF is cloned in a mammalian expression cassette consisting of an ubiquitous EF-1 α composite promoter and the strong SV40 polyadenylation signal.

Selectable with Ampicillin or the Selection of Your Choice

pORF plasmids are selectable in bacteria with ampicillin. We can replace the bacterial selection cassette with a mammalian selection cassette expressing the resistance gene of your choice (Blasti[®], Hygro[®], Neo[®], Puro[®], Zeo[®], GFP::Zeo[®], Luc::Zeo[®]).

Selection of InvivoGen's Immune Genes

CD Antigens	B7.1, B7.2, CD70, ICAM-1, LFA-3
Chemokines	MCP-1, MIP-1, MIP-3, SDF-1 α , RANTES
Cytokines	G-CSF, GM-CSF, VIP
Cytokine Suppressors	SOCS-1, -2, -3, -4
Cytokine Receptors	IL-1R, IL-8R, IL-10R, IL-13R, TNF-R
Interferons	IFN-alpha, -beta, -gamma, -tau
Interleukins	IL-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11 IL-12, -13, -14, -15, -16, -17, -18BP, -21 IL-22, -23, -24, -30
TNF Superfamily	4-1BBL, CD40L, FasL, OX40L, TNF α , TRAIL, TRANCE

For a complete list, visit

<http://www.invivogen.com/plasmids/pORF.htm>



Product	Quantity	Price
pORF-<Gene>	<i>E. coli</i> disk	\$395
pORF-<Fusion Gene>	<i>E. coli</i> disk	\$435
pORF-mcs	<i>E. coli</i> disk	\$200-\$265
Custom plasmid	20 μ g	from \$800

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