

InvivoGen Insight

An Insightful Look At InvivoGen's Innovative Products

Toll-like Receptors (TLRs) play a critical role in the detection of invading pathogens and the induction of host defense mechanisms. Recently, an eleventh TLR has been identified. This mouse **TLR11** recognizes uropathogenic *E. coli* strains and seems to be involved in the prevention of urogenital infections. InvivoGen offers this newly cloned TLR gene in a pUNO plasmid, as well as heat-killed uropathogenic *E. coli* cells (HKUEC) that are recognized by TLR11. New TLR ligands have also been added to our extensive list, such as **MALP2**, *P. gingivalis* LPS and single stranded RNAs, the natural ligands of TLR7 and TLR8.

Detection of TLR expression by Western blot analysis has been limited by the quality and availability of TLR antibodies. To overcome this problem, InvivoGen has engineered **HA-tagged TLR genes** that are readily detectable with a HA monoclonal antibody (mAb).

Immunoconjugates that combine a mAb and a toxic drug, such as geldanamycin, represent a promising approach to treat some types of solid tumors. InvivoGen provides **GMB-APA-GA**, a pre-conjugated form of geldanamycin.

Lastly, InvivoGen offers the largest collection of mammalian promoters, either ubiquitous or specific, cloned in the pDRIVE plasmid.

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Toll-like Receptor 11: A New Member of the TLR Family

To date, ten mammalian Toll-like receptors (TLRs) have been identified. They recognize specific molecular patterns associated with microbial pathogens and induce the activation of host-defense genes. Recently, with the completion of the human and mouse genome sequence, an eleventh TLR has been identified. Using the TIR domain of TLR4 to search the NCBI databases, Zhang *et al.* have detected the sequence of TLR11 from a mouse liver EST database (GenBank accession number AY531552). This gene encodes a 97-kD protein which displays all the hallmarks of known TLRs, that is a leucine-rich domain, a transmembrane domain and a TIR domain.

TLR11 is highly expressed in the liver, bladder and kidney but weakly in the spleen, a pattern that differs from the other TLRs, suggesting that TLR11 might play a specific role in these organs. Zhang *et al.* hypothesized that TLR11 might be involved in responses to bacteria that cause infections of the urinary tract, such as uropathogenic *Escherichia coli*. Various uropathogenic strains were cultivated then heat-killed and used to stimulate 293-luciferase cells stably expressing TLR11. The uropathogenic strains induced a strong activation of NF- κ B in these cells in contrast to the nonpathogenic strains such as *E. coli* BL21 and DH5 α . Known TLR ligands, including lipopolysaccharide (LPS), peptidoglycan (PGN) and poly(I:C), failed to activate NF- κ B in the TLR11-expressing 293 cells. These data suggest that TLR11 recognizes a molecular pattern that is specific to *E. coli* uropathogenic strains.

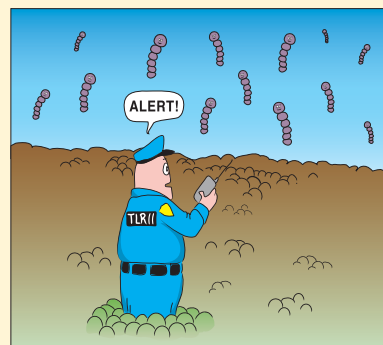
TLR11 activates the common TLR signaling pathway that involves the intermediates MyD88, IRAK and TRAF6². TLR11 sequences are present in the genomes of most mammalian species including humans. However, the putative TLR11 ORF contains several Stop codons suggesting that TLR11 might not be expressed in humans.

To further study the biological role of this new TLR, mice deficient in TLR11 were generated. Macrophages isolated from these mice were stimulated with known TLR ligands (LPS, PGN, poly(I:C)), and heat-killed

uropathogenic or nonpathogenic *E. coli*. All three TLR ligands induced the production of TNF- α in TLR11-deficient and wild-type macrophages at similar levels. In contrast, the response to the uropathogenic strain was dramatically reduced in the knock-out macrophages compared to the wild-type cells, whereas the response to the nonpathogenic strain was almost unaffected. These results confirm that TLR11 recognizes a component in the uropathogenic *E. coli* that is absent from the nonpathogenic strain. The genome of uropathogenic *E. coli*, such as CFT073 (ATCC 700928) is 590 Kbp longer than the genome of nonpathogenic and laboratory *E. coli* strains and is particularly rich in genes that encode virulence factors such as fimbrial adhesins and autotransporters³. One of these microbial elements could be the molecular pattern recognized by TLR11.

InvivoGen provides the murine TLR11 gene in a pUNO plasmid (# puno-mtlr11) as well as heat-killed cells of the uropathogenic strain CFT073 (# tlr11-hkuec).

1. Zhang D. *et al.*, 2004. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science*. 303:1522-1526.
2. O'Neill LA. 2002. Signal transduction pathways activated by the IL-1 receptor/toll-like receptor superfamily. *Curr Top Microbiol Immunol*. 270:47-61. Review.
3. Welch RA. *et al.*, 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA*. 99(26):17020-4.



Toll-Like Receptors

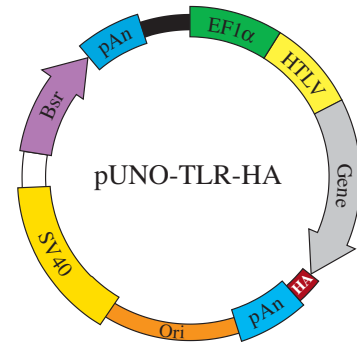
Toll-like receptors (TLRs) are type I transmembrane receptors that recognize specific pathogen associated molecular patterns (PAMPs). These PAMPs, aka TLR ligands, induce various signaling pathways leading to distinct immune responses. To help you unravel the mechanisms activated by the TLRs or exploit their tremendous potential in human therapy, InvivoGen strives to provide the most comprehensive TLR-related product line which includes new TLR ligands, more 293/TLR clones and easy-to-detect TLR genes.

pUNO-TLR-HA

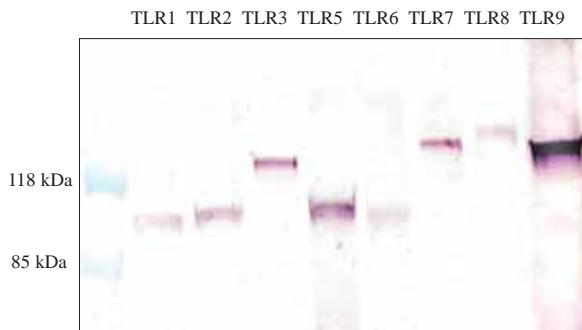
HA-tagged TLR genes

pUNO-TLR-HA is a family of expression vectors featuring HA-tagged TLR genes. All human and murine TLR genes have been fused at the 3' end to the influenza hemagglutinine (HA) tag. This short sequence (YPYDVPDYA) encodes a peptide which is the epitope of a very efficient and specific monoclonal antibody. The use of HA-tagged TLR genes provides a simple and convenient method to detect the expression of the TLR genes by Western blot. All TLR genes can be detected using the same primary antibody.

pUNO-TLR-HA plasmids carry a blasticidin resistance gene for amplification in *E. coli* and selection in mammalian cells. Blasticidin is a potent antibiotic that allows the selection of stable mammalian clones in as little as one week.



- Detection of all TLR genes with a single antibody
- Fully functional TLR genes
- High-levels of expression



| Gene | Code (human) | Code (mouse) | Quantity | Price |
|----------|---------------|---------------|----------|-------|
| TLR1-HA | puno-htrl1ha | puno-mtrl1ha | 1 disk | \$445 |
| TLR2-HA | puno-htrl2ha | puno-mtrl2ha | 1 disk | \$445 |
| TLR3-HA | puno-htrl3ha | puno-mtrl3ha | 1 disk | \$445 |
| TLR4-HA | puno-htrl4ha | puno-mtrl4ha | 1 disk | \$445 |
| TLR5-HA | puno-htrl5ha | puno-mtrl5ha | 1 disk | \$445 |
| TLR6-HA | puno-htrl6ha | puno-mtrl6ha | 1 disk | \$445 |
| TLR7-HA | puno-htrl7ha | puno-mtrl7ha | 1 disk | \$445 |
| TLR8-HA | puno-htrl8ha | puno-mtrl8ha | 1 disk | \$445 |
| TLR9-HA | puno-htrl9ha | puno-mtrl9ha | 1 disk | \$445 |
| TLR10-HA | puno-htrl10ha | | 1 disk | \$445 |
| TLR11-HA | | puno-mtrl11ha | 1 disk | \$445 |



Related products:

Anti-HAtag Mouse monoclonal #ab-hatag 250 µl \$175

293/TLR-CD14 Clones

CD14, a GPI-anchored coreceptor, is often required for optimal responses to TLR2, TLR4 and TLR5 ligands, such as LTA, LPS and flagellin.

293/TLR-CD14 clones are HEK293 cells transfected with a pUNO-TLR or pDUO-TLR plasmid. Expression of the TLR and coreceptor genes has been verified using a functional assay.

293/TLR-CD14 clones are grown in standard DMEM medium with 10% FBS supplemented with 10 µg/ml blasticidin and/or 100 µg/ml HygroGold (hygromycin B). Each vial of 293/TLR-CD14 clone contains 1-5 x 10⁶ cells and is shipped on dry ice.

| Product | Code | Price |
|--------------------|------------------|--------|
| 293/hMD2-CD14 | 293-hmd2cd14 | \$800 |
| 293/hTLR2-CD14 | 293-htrl2cd14 | \$1000 |
| 293/hTLR4-MD2-CD14 | 293-htrl4md2cd14 | \$1200 |
| 293/hTLR5-CD14 | 293-htrl5cd14 | \$1000 |
| 293/LacZ (control) | 293-lacz | \$600 |

Related products:

Blasticidin (100 mg) ant-bl-1 \$180
HygroGold (1 g) ant-hg-1 \$100

Geldanamycin Immunoconjugate

Geldanamycin (GA) is an ansamycin benzoquinone antibiotic in development as a lead anticancer drug. GA exerts its cytotoxicity by inhibiting the chaperone function of the heat shock protein 90 (Hsp90), leading to the depletion of multiple oncogenic client proteins¹. While the antitumor potential of GA has long been recognized, clinical use of native GA has not been pursued due to the severe toxicity of the drug.

Reduction of GA systemic toxicity could potentially be achieved by selectively targeting and delivering an active GA species into malignant cells using a monoclonal antibody (mAb) as the targeting vehicle. Several tumor-targeting mAb armed with small toxic compounds have already been clinically evaluated, and one, Mylotarg, has been approved for the treatment of acute myeloid leukemia.

Herceptin, the first mAb approved for therapy of solid tumors, targets Her2 and was chosen to target GA to Her2-overexpressing tumors. NCI has reported that such conjugates deliver a more potent selective cytotoxic impact than Herceptin alone².

To prepare such conjugates, GA is modified to introduce a latent primary amine³. After deprotection, this primary amine provides a site for introduction of a maleimide group that enables linkage to proteins.

TLR Ligands

• TLR2 Ligands

Heat-killed *P. gingivalis* (HKPG) - HKPG is a freeze-dried heat-killed preparation of the periodontopathic bacteria *Porphyromonas gingivalis*. In Chinese hamster ovary (CHO) cells cotransfected with CD14 and TLR2 exposure to HKPG induces the activation of NF- κ B dose dependently indicating that TLR2 plays an important role in the recognition of these bacteria. Expression of TLR4 failed to enhance the response to HKPG suggesting that either the whole bacterial components of *P. gingivalis* are not recognized by TLR4 or some components of these bacteria inhibit TLR4-mediated activation¹.

Working concentration: 10⁸ cells/ml

LPS from *P. gingivalis* - Recognition of LPS from *P. gingivalis*, a Gram-negative bacteria, is mediated by TLR2 and CD14, and unlike enteric LPS, is able to induce a septic shock in C3H/HeJ mice which are deficient for TLR4 and hyporesponsive to *E. coli* LPS. This property is attributed mainly to the unique lipid A motif of *P. gingivalis* LPS which contains unusually branched and relatively long fatty acids. This LPS was reported to inhibit TLR4-mediated signaling but not TLR2-mediated signaling^{1,2}. At low concentrations (<1 μ g/ml), *P. gingivalis* LPS induces TLR2, and at higher concentrations (\geq 10 μ g/ml), it induces strongly TLR2 and to a lesser extent TLR4.

Working concentration: 10 ng - 10 μ g/ml

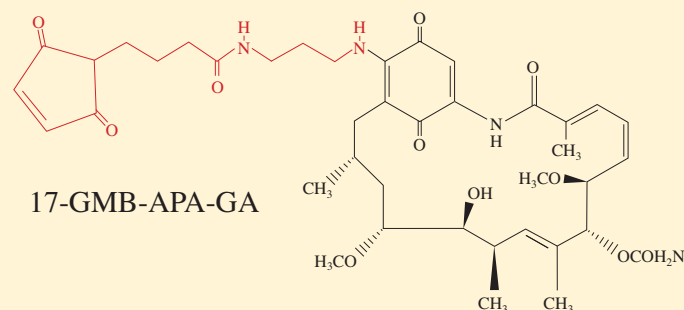
• TLR2/6 Ligand

MALP2 - Natural macrophage-activating lipopeptide 2 (MALP-2), originally isolated from the membrane of *Mycoplasma fermentans*, and its synthetic derivative are potent activators of macrophages. Mycoplasma lipopeptides (LP), such as MALP2, are diacylated and recognized by TLR2 and TLR6, whereas bacterial LP and Pam3CSK4, which are triacylated, are recognized by TLR2 and TLR1³. MALP2 stimulation induces a MyD88-dependent signaling cascade leading to the activation of NF- κ B and the production of proinflammatory cytokines.

Working concentration: 10 ng - 1 μ g/ml

InvivoGen provides this maleimido derivative of geldanamycin, 17-(3-(4-maleimidobutylcarboxamido)propyl-amido) geldanamycin (GMB-APA-GA), which is ready to be conjugated with Herceptin or other mAbs. Catalog code: gmbapa-ga (1 mg) - Inquire for pricing or check our website.

1. Neckers L. *et al.*, 1999. Geldanamycin as a potential anti-cancer agent: its molecular target and biochemical activity. *Invest. New Drugs* 17:361-373.
2. Mandler R. *et al.*, 2004. Herceptin-geldanamycin immunoconjugates: pharmacokinetics, biodistribution, and enhanced antitumor activity. *Cancer Res.* 64(4):1460-7.
3. Mandler R. *et al.*, 2002. Modifications in synthesis strategy improve the yield and efficacy of geldanamycin-herceptin immunoconjugates. *Bioconjug Chem.* 13(4):786-91.



• TLR7/8 Ligands

Single stranded RNAs (ssRNAs) - Recently, two distinct teams have identified single-stranded RNA (ssRNA) as the natural ligand of TLR7 and TLR8^{4,5}. ssRNA derived from HIV-1 or the influenza virus were shown to induce the production of proinflammatory cytokines in PDC. This induction was reproduced using GU-rich (ssRNA33 & ssRNA40)⁴ or polyU⁵ oligonucleotides complexed with cationic lipids to facilitate their uptake. Upon stimulation with ssRNA, murine TLR7 and human TLR8 induced the activation of NF- κ B whereas human TLR7 and murine TLR8 failed, implying a species specificity difference in ssRNA recognition. ssRNA-induced TLR7/8 signaling was abrogated by chloroquine, indicating that it is dependent on endosomal acidification. TLR7/8, which can recognize both self and viral RNA, seem to detect the abnormal localization of ligands rather than molecular patterns absent from the host. Thus complexed GU-rich or polyU oligonucleotides may represent potent adjuvants for vaccination and immunotherapy.

Working concentration: 0.1-10 μ g/ml

• TLR11 Ligand

Heat-killed uropathogenic *E. coli* (HKUEC) - TLR11 is the newest member of the TLR family. Mouse TLR11 recognizes uropathogenic *E. coli* strains and induces the common MyD88-dependent TLR signaling pathway⁶. HKUEC is a freeze-dried heat-killed preparation of the uropathogenic *E. coli* strain CFT073 (ATCC 700928).

Working concentration: 10⁸ cells/ml

• Related Products

Polymixin B (10 μ g/ml) can be used to block LPS-induced activation of TLR4⁷. **Chloroquine** (10 μ M) can be used to block endosomal acidification⁸.

1. Yoshimura A. *et al.*, 2002. Lipopolysaccharides from periodontopathic bacteria *Porphyromonas gingivalis* and *Capnocytophaga ochracea* are antagonists for human toll-like receptor 4. *Infect Immun.* 70(1):218-25.
2. Coats SR. *et al.*, 2003. *Porphyromonas gingivalis* lipopolysaccharide antagonizes *Escherichia coli* lipopolysaccharide at toll-like receptor 4 in human endothelial cells. *Infect Immun.* 71(12):6799-807.
3. Takeda K. *et al.*, 2002. Recognition of lipopeptides by Toll-like receptors. *J Endotoxin Res.* 8(6):459-63.
4. Heil F. *et al.*, 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science.* 5:303(5663):1526-9.
5. Diebold SS. *et al.*, 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science.* 5:303(5663):1529-31.
6. Zhang D. *et al.*, 2004. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science.* 303:1522-1526.
7. Becker S. *et al.*, 2002. Involvement of microbial components and toll-like receptors 2 and 4 in cytokine responses to air pollution particles. *Am J Respir Cell Mol Biol.* 27(5):611-8.

| Product | Code | Quantity | Price |
|--|-------------|------------------------|-------|
| TLR2 ligands | | | |
| Heat-killed <i>P. gingivalis</i> (HKPG) | ttrl-hkpg | 10 ¹⁰ cells | \$90 |
| Ultrapure LPS from <i>P. gingivalis</i> | ttrl-pglps | 1 mg | \$140 |
| TLR2/6 ligand | | | |
| MALP2 | ttrl-mlp | 100 μ g | \$240 |
| TLR7 / TLR8 ligands | | | |
| Lyophilized ssPolyU/LyoVec | ttrl-lpu | 100 μ g | \$160 |
| Lyophilized ssRNA33/LyoVec | ttrl-lrna33 | 100 μ g | \$240 |
| Lyophilized ssRNA40/LyoVec | ttrl-lrna40 | 100 μ g | \$240 |
| TLR11 ligand | | | |
| Heat-killed uropathogenic <i>E. coli</i> | ttrl-hkuec | 10 ¹⁰ cells | \$100 |

Related products:

| | | | |
|-------------|----------|--------|-------|
| Chloroquine | ttrl-chq | 250 mg | \$100 |
| Polymixin B | ttrl-pmb | 100 mg | \$100 |

pDRIVE

A Comprehensive Collection of Mammalian Promoters

pDRIVE is a family of expression vectors that feature an expanding list of human and rodent promoters. Each promoter drives the expression of the LacZ reporter gene which allows for testing of the promoter activity in transient transfection experiments. The promoter of interest and the lacZ gene are flanked by unique restriction sites making their replacement or subcloning easy.

Ubiquitous or Specific Promoters

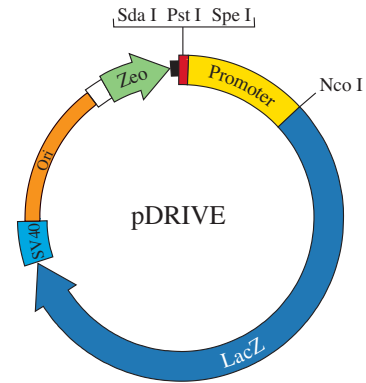
InvivoGen offers a large choice of ubiquitous promoters that feature a strong activity in a wide variety of cells, tissues and cell cycles (Fig 1A & 1B), as well as specific promoters that drive gene expression in specific types of cells or tissues (Fig 2A & 2B).

Native or Composite Promoters

InvivoGen provides native unmodified promoters that comprise in a single DNA fragment the core promoter and 5'UTR of a given gene. InvivoGen has also engineered composite promoters by combining several DNA fragments that contain a core promoter, a 5'UTR and/or an enhancer from different origins. These composite promoters display a stronger activity than their native counterparts and in some cases a different expression pattern.

Well-characterized Promoters

Each promoter has been fully sequenced. Most of them have been tested in a variety of cell lines to confirm their specific expression pattern and compare their activity to commonly used promoters.



Selection from InvivoGen's Promoter List

| Promoter | Name | Species | Expression Pattern |
|----------|---------------------------------|--------------|--------------------------|
| AFP | Alpha-Fetoprotein | Human | Hepatocellular carcinoma |
| B29 | Immunoglobulin Beta | Human, mouse | B cells |
| CAG | CMV enh/ β-actin prom | Chicken | Ubiquitous |
| COX2 | Cyclo-oxygenase 2 | Human, mouse | Tumor |
| EF-1α | Elongation factor 1 alpha | Mouse, rat | Ubiquitous |
| Flt-1 | VEGF receptor 1 | Human | Endothelial cells |
| GFAP | Glial fibrillary acidic protein | Human, rat | Astrocytes |
| HSP70 | Heat shock protein 70 | Human, mouse | Ubiquitous, heat-induced |
| Mb | Myoglobin | Human, mouse | Muscle |
| NSE | Neuron specific enolase | Rat | Mature neurons |
| OG-2 | Osteocalcin 2 | Mouse | Osteoblasts |
| PGK1 | Phosphoglycerate kinase 1 | Mouse | Ubiquitous |
| PSA | Prostate specific antigen | Human | Prostate |
| ROSA | ROSA 26 | Mouse | Ubiquitous |
| Tyr | Tyrosinase | Mouse | Melanocytes, melanoma |

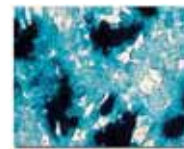


Fig 1A: Human 293 cells transfected with pDRIVE-rEF1 carrying the rat EF1α promoter



Fig 2A: 293 cells transfected with pDRIVE-SV40-hAFP carrying the SV40 enh/alpha-fetoprotein promoter

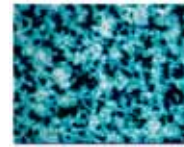


Fig 1B: Mouse B16 cells transfected with pDRIVE-rEF1 carrying the rat EF1α promoter

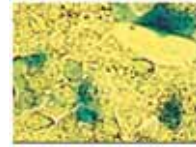


Fig 2B: HepG2 cells transfected with pDRIVE-SV40-hAFP carrying the SV40 enh/alpha-fetoprotein promoter

| Product | Quantity | Price |
|------------------------|---------------------|------------|
| pDRIVE<native prom> | <i>E. coli</i> disk | \$430 |
| pDRIVE<composite prom> | <i>E. coli</i> disk | \$470 |
| pDRIVE-CMV | <i>E. coli</i> disk | \$430 |
| pDRIVE-Custom | 20 µg | From \$800 |

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