As summer approaches, mycoplasma contamination of cell cultures is becoming an alarming concern. For the past five years, InvivoGen has provided Plasmocin™ that is now recognized as the most efficient mycoplasma removal agent allowing you to save your valuable cell lines. InvivoGen continues to expand its collection of TLR ligands. In this issue you will find new ligands for TLR2 and TLR7/8, labeled ODNs for TLR9 and a kit that contains a ligand for each TLR. In addition, we provide a set of RT-PCR primers to detect the expression of human TLRs. The featured product in this newsletter is pCpG, a CpG-free plasmid designed for sustained expression in vivo. Lastly, we provide pFUSE-Fc the first plasmid commercially available to facilitate the construction of Fc-fusions. More information regarding these products is available on our redesigned website launched a few weeks ago. Check our new website and get 20% off your first online order.

**Inside this issue:**

- TLR Ligands
  - Purified LTA
  - Pam2CSK4 - Synthetic LP
  - Recombinant Flagellin
  - Single-stranded RNA-DR
  - FITC-labeled CpG-ODNs
  - TLR Agonist Kit

- TLR Expression
  - TLR RT-Primers

- CpG-free Plasmids
  - Long lasting expression in vivo

- Fc Fusions
  - pFUSE-Fc

- Mycoplasma Eradication
  - Plasmocin™

---

### Mycoplasma Eradication

Mycoplasma contamination of cultured cells is a major problem in both basic research and industrial production. Up to 87% of cell lines may be contaminated by mycoplasma. Mycoplasma infection can affect virtually any function and activity of eukaryotic cells leading to experimental artifacts and unreliable results.

It is usually recommended that an infected cell culture be immediately autoclaved to prevent the infection from spreading and to use only mycoplasma-free cultures. However, some cell lines are irreproducible and require an effective eradication treatment. The use of specific antibiotics can efficiently eliminate mycoplasma contaminations. Antibiotics commonly used in cell culture are inactive on mycoplasma (e.g. penicillins and streptomycin). Three classes of antibiotics have been shown to kill mycoplasma at relatively low concentrations: tetracyclines, macrolides and quinolones. Tetracyclines and macrolides block the protein synthesis by interfering with ribosome translation, while quinolones inhibit the replication of bacterial DNA.

Several antibiotics are commercially available for the removal of mycoplasma: BM-Cyclin (Roche) contains a macrolide and a tetracycline, Ciprofloxin (Bayer, available only with a prescription) and MRA (ICN) are both quinolones. Plasmocin™ (InvivoGen) is the only antimycoplasma reagent that combines a macrolide and a quinolone. Unlike BM-Cyclin that requires the sequential and cyclic use of two antibiotics, Plasmocin™ is ready-to-use and can be added to the culture medium directly. Furthermore, both components in Plasmocin™ act on separate targets blocking protein synthesis and DNA replication, whereas the two antibiotics in BM-Cyclin are both inhibitors of protein synthesis. Therefore, Plasmocin™ is more effective in removing mycoplasma and prevents the appearance of resistant strains. In contrast to other anti-mycoplasma compounds, Plasmocin™ is active on both free mycoplasma as well as intracellular forms. This advantage is conferred by one component of Plasmocin™ which is actively transported into mammalian cells. It ensures that following treatment with Plasmocin™ a cell culture is not reinfected by mycoplasma released from intracellular compartments of infected cells. To date, no consistent and permanent alterations that affect the eukaryotic cells during and after the treatment have been detected1.

---

### Plasmocin™ Kills Mycoplasma

![Plasmocin™ Kills Mycoplasma](image)

Plasmocin™ is ready-to-use and can be added to the culture medium directly. Furthermore, both components in Plasmocin™ act on separate targets blocking protein synthesis and DNA replication, whereas the two antibiotics in BM-Cyclin are both inhibitors of protein synthesis. Therefore, Plasmocin™ is more effective in removing mycoplasma and prevents the appearance of resistant strains. In contrast to other anti-mycoplasma compounds, Plasmocin™ is active on both free mycoplasma as well as intracellular forms. This advantage is conferred by one component of Plasmocin™ which is actively transported into mammalian cells. It ensures that following treatment with Plasmocin™ a cell culture is not reinfected by mycoplasma released from intracellular compartments of infected cells. To date, no consistent and permanent alterations that affect the eukaryotic cells during and after the treatment have been detected1.


### Comparison of the most common anti-mycoplasma agents

<table>
<thead>
<tr>
<th>Product</th>
<th>Supplier</th>
<th>Treatment</th>
<th>Ease of use</th>
<th>Efficacy</th>
<th>Cytotoxicity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-Cyclin</td>
<td>Roche</td>
<td>3 weeks</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Ciprofloxin</td>
<td>Bayer</td>
<td>12 to 20 days</td>
<td>+</td>
<td>++</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>MRA</td>
<td>ICN</td>
<td>1 to 2 weeks</td>
<td>+</td>
<td>++</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Plasmocin</td>
<td>InvivoGen</td>
<td>2 weeks</td>
<td>+</td>
<td>+++</td>
<td>+/-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Plasmocin for treatment:** Catalog code: ant-mpt - Quantity: 50 mg (treats 2 liters) - Price: $125
**TLR Ligands**

- **TLR2 Ligand - Purified LTA-SA**
  Lipoteichoic acid (LTA) is a major immunostimulatory component of Gram-positive bacteria. This new preparation of LTA from *S. aureus* (LTA-SA) is purified following the method described by Morath et al. It contains 10 times less endotoxin according to the gel clot LAL Assay than the standard preparation. At concentrations ranging from 10 ng to 10 µg/ml, it highly activates TLR2 and no other TLRs including TLR4.
  **Working concentration:** 10 ng - 10 µg/ml

- **TLR2/6 Ligand - Pam2CSK4**
  Pam2CSK4 is a synthetic diacylated lipopeptide (LP). Bacterial lipoproteins are strong immune modulators that activate early innate host responses after infection. LP analogues of these lipoproteins signal either through TLR2/1 or TLR2/6 heterodimers. According to the current model, triacylated LP like Pam3CSK4, are recognized by TLR2/1, whereas diacylated LP, such as FSL1, induce signaling through TLR2/6. However, it was recently reported that diacylated LP, such as Pam2CSK4, induce signaling in a TLR6-independent manner. This finding suggests that both the lipid and peptide part of lipoproteins take part in the specificity of recognition by TLR2 heterodimers.
  **Working concentration:** 1-100 ng/ml

- **TLR5 Ligand - Recombinant Flagellin from *S. typhimurium***
  Flagellin is the major component of the bacterial flagellar filament and a potent stimulator of innate immune responses. *FliC* is one of the genes known to encode flagellin in *Salmonella*. The gene was expressed in HEK293 cells and the protein purified. The resulting recombinant flagellin is endotoxin-free according to the gel clot LAL Assay.
  **Working concentration:** 10 ng - 1 µg/ml

- **TLR7/8 Ligand - Single stranded RNA Double-Right (ssRNA-DR)**
  Long double stranded RNA (>30 bp) are known to induce type I interferons (IFNs) while siRNAs are thought to be short enough to bypass this immune response. However, Hornung et al. recently reported that certain siRNA sequences were potent stimuli of IFN-α. This stimulation is sequence-dependent and is triggered by the sense strand. The putative immunostimulatory motif was identified as a 9 mer sequence (5’-GUCCUUCAA-3’) which when placed twice in a 19 mer RNA oligonucleotide (ssRNA-DR) was shown to be more immunostimulatory than when present as one copy. This sequence-specific recognition is mediated by TLR7/8, a sensor of ssRNA viruses. ssRNA-DR is provided pre-complexed with the cationic lipid LyoVec™ to facilitate its uptake.
  **Working concentration:** 1-10 µg/ml

**References:**

**Labeled Ligands**

- **TLR9 Ligands - FITC CpG-ODNs**
  Unmethylated CpG-ODNs are recognized by TLR9 which is expressed in the endoplasmic reticulum. ODN 1826 (mouse) and ODN 2006 (human, type B) have been labeled with FITC at their 5’ terminus. FITC-labeled CpG-ODNs are useful to study their cellular uptake and localization by confocal laser-scanning microscopy or flow cytometry.
  **Working concentration:** 5 µM / 6 µg/ml

**References:**

**Product** | **Quantity** | **Code** | **Price**
--- | --- | --- | ---
TLR2 ligand - Purified LTA-SA | 5 mg | tlr-lpsla | $140
TLR2/6 ligand - Pam2CSK4 | 100 µg | tlr-pam2 | $200
TLR5 ligand - Recombinant Flagellin | 1 µg | tlr-flic | $190
TLR7/8 ligand - ssRNA-DR | 100 µg | tlr-ssdr | $240
TLR1-9 ligands - TLR Agonist Kit | - | tlr-kit1 | $380
Labeled ligands - FITC-CpG ODNs - FITC ODN1826 | 50 µg | tlr-fmodn | $110
- FITC ODN2006 | 50 µg | tlr-fhodnb | $110

**U.S. Contact:**  Toll Free  888.457.5873  •  Fax  858.457.5843  •  Email  info@invivogen.com
TLR Expression

TLR RT-Primers

Toll-like Receptors are predominantly expressed in tissues involved in immune function, such as spleen and peripheral blood leukocytes, as well as those exposed to the external environment such as lung and the gastrointestinal tract. Their expression profiles vary among tissues and cell types.

InvivoGen provides TLR RT-Primers, a collection of RT-PCR primers to determine the mRNA expression pattern of human TLRs. They can be used to analyze the expression of endogenous as well as transgenic human TLR genes. Each TLR RT-Primer pair is carefully designed and tested. TLR RT-Primers are provided as pairs for each individual TLR or as a set containing a primer pair for all ten human TLRs. The size of the amplified fragments varies from 200 to 700 bp.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Code</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR RT-Primer Pairs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR1</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl1</td>
<td>$110</td>
</tr>
<tr>
<td>TLR2</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl2</td>
<td>$110</td>
</tr>
<tr>
<td>TLR3</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl3</td>
<td>$110</td>
</tr>
<tr>
<td>TLR4</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl4</td>
<td>$110</td>
</tr>
<tr>
<td>TLR5</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl5</td>
<td>$110</td>
</tr>
<tr>
<td>TLR6</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl6</td>
<td>$110</td>
</tr>
<tr>
<td>TLR7</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl7</td>
<td>$110</td>
</tr>
<tr>
<td>TLR8</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl8</td>
<td>$110</td>
</tr>
<tr>
<td>TLR9</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl9</td>
<td>$110</td>
</tr>
<tr>
<td>TLR10</td>
<td>2.5 nmol each</td>
<td>rtp-hlrl10</td>
<td>$110</td>
</tr>
<tr>
<td>TLR RT-Primer Set</td>
<td>2.5 nmol each</td>
<td>rts-hlrs</td>
<td>$480</td>
</tr>
</tbody>
</table>

CpG-free Plasmids

Long lasting expression in vivo

The success of gene therapy will require long lasting expression of the transgene. One of the major limitations to sustained transgene expression is the immunostimulatory effects of the plasmid DNA (pDNA). Indeed, pDNA carry elements of bacterial (origin of replication, resistance gene) and viral (promoter) origins that contain unmethylated CpG motifs known to rapidly induce the innate immune system through TLR9. To minimize the immunostimulatory effects of pDNA, InvivoGen has developed pCpG, a family of revolutionary plasmids that are completely devoid of CpGs. pCpG plasmids were generated by assembling elements that either naturally lack CpG dinucleotides, were modified to remove all CpGs, or chemically synthesized. pCpG plasmids feature a strong and ubiquitous mammalian promoter that combines CpG-free versions of the mouse CMV enhancer and the human EF1 promoter (mCMV/hEF1). In vivo experiments using the hydrodynamic method revealed that the mCMV/hEF1 promoter was as strong as the widely used human CMV promoter. However, in marked contrast to the CMV promoter which expression lasted less than 2 weeks due to extensive methylation, the pCpG composite promoter allowed sustained and stable expression of the transgene over 2 months. In addition to its lack of immunogenicity, the long lasting expression of pCpG plasmids is believed to be due to its episomal status conferred by the presence of two scaffold/matrix attachment regions.

Expression levels of pCpG and CMV-based plasmids - Nine week old mice (5 mice/group) were injected with 30 μg pCpG-mSEAP or pcDNA3-mSEAP, a CMV-based plasmid (Invitrogen), using the hydrodynamic method. Both plasmids express the mouse secreted embryonic alkaline phosphatase (mSEAP). The activity of mSEAP present in the sera was measured over 60 days with the SEAP Detection Kit (cat. code #rep-sap).

Expression of TLR mRNAs in human monocytic cell line THP-1 using the TLR RT-Primer Set.
**pFUSE-Fc**

Fc Fusions Made Easy

Fc-fusion proteins are widely used to promote long-lasting expression of a protein of interest *in vivo*. They are generated by fusing the Fc region of an immunoglobulin to their C terminus. These soluble chimeras retain the activity of the native protein and present, in addition to a long half-life in the circulatory system, the advantages of efficient mammalian expression and ease of purification. Fc-fusion proteins are useful research tools for many applications and hold promise as therapeutics. InvivoGen provides pFUSE-Fc, a new expression plasmid, designed to facilitate the generation of Fc-fusion proteins.

- **pFUSE-Fc** plasmids yield high levels of Fc-Fusion proteins. The level of expression is usually in the µg/mL range.
- **pFUSE-Fc** plasmids feature several Fc regions from various species:
  - human Fc from IgG1
  - mouse Fc from IgG2a
  - rabbit Fc from IgG
- **pFUSE-Fc** plasmids can be transfected in a variety of mammalian cells commonly used in protein purification systems, including CHO, COS, HEK293 and myeloma cell lines.
- **pFUSE-Fc** plasmids are selectable with Zeocin™ in *E. coli* as well as in mammalian cells. Thus, they can be used to transfect mammalian cells transiently or stably.
- **pFUSE-Fc** plasmids allow the secretion of Fc-Fusion proteins.
  - **pFUSE-Fc1** is recommended when the protein of interest contains a native signal sequence.
  - **pFUSE-Fc2** contains an IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted.
- **Fc-Fusion proteins can be easily detected** in the supernatant of pFUSE-Fc-transfected cells by SDS-PAGE. Their functional domains can be identified by immunoblotting and ligand blotting.
- **Fc-Fusion proteins can be easily purified** by single-step protein A or protein G affinity chromatography.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Code</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFUSE-hFc1</td>
<td>20 µg</td>
<td>pfuse-hfc1</td>
<td>$435</td>
</tr>
<tr>
<td>pFUSE-hFc2 (IL2ss)</td>
<td>20 µg</td>
<td>pfuse-hfc2</td>
<td>$435</td>
</tr>
<tr>
<td>pFUSE-mFc1</td>
<td>20 µg</td>
<td>pfuse-mfc1</td>
<td>$435</td>
</tr>
<tr>
<td>pFUSE-mFc2 (IL2ss)</td>
<td>20 µg</td>
<td>pfuse-mfc2</td>
<td>$435</td>
</tr>
<tr>
<td>pFUSE-rFc1</td>
<td>20 µg</td>
<td>pfuse-rfc1</td>
<td>$435</td>
</tr>
<tr>
<td>pFUSE-rFc2 (IL2ss)</td>
<td>20 µg</td>
<td>pfuse-rfc2</td>
<td>$435</td>
</tr>
</tbody>
</table>