The response is mediated by TLR2 and TLR3. Stimulation with HKLM induces NF-κB to induce the signaling cascade leading to the activation of NF-κB, characterized by the secretion of proinflammatory cytokines. This proinflammatory cytokine production is mediated by TLR2, which cooperates with TLR1 through their cytoplasmic domain to induce the signaling cascade leading to the activation of NF-κB.

- **HKLM** is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces a strong nonspecific response in a number of eukaryotic cells and organisms, including both mammals and plants. Flagellin induces the immediate activation of NF-κB and the production of proinflammatory cytokines.

- **Pam3CSK4** is a synthetic tripalmitoylated lipopeptide that mimics the acylated amino terminus of bacterial lipoproteins. Pam3CysSerLys4 (Pam3CSK4) is a potent activator of the proinflammatory signaling pathway. LPS signaling also involves a MyD88-independent pathway. LPS binding to its receptor, TLR4, triggers the production of proinflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes via the adaptor TRIF.

- **E. coli K12 LPS** - TLR4 agonist
  Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. LPS recognition is mediated by TLR4 which forms a complex with MD2 and CD14 leading the production of proinflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes via the adaptor TRIF.

- **S. typhimurium Flagellin** - TLR5 agonist
  Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. Flagellin is a potent stimulator of innate immune responses in a number of eukaryotic cells and organisms, including both mammals and plants. In mammals, flagellin is recognized by TLR5 and triggers defense responses both systemically and at epithelial surfaces. Flagellin induces the activation of NF-kB and the production of cytokines and nitric oxide depending on the nature of the TLR5 signaling complex.

- **FSL-1** - TLR6/2 agonist
  FSL-1 (Pam2CGDPKHPKSF) is a synthetic lipoprotein that represents the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma fermentans*. The framework structure of FSL-1 is the same as that of MALP-2, a *Mycoplasma salivarium* derived lipopeptide (LP), but they differ in the amino acid sequence and length of the peptide portion. FSL-1 is recognized by TLR2 and TLR6 inducing a MyD88-dependent signaling cascade that leads to the activation of NF-kB and the production of proinflammatory cytokines.

- **Poly(I:C) and Poly(I:C) LMW** - TLR3 agonists
  Poly(I:C) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Poly(I:C) is composed of a strand of poly(I) annealed to a strand of poly(C). The size of the strands varies. Poly(I:C) has a high molecular weight (average size 1.5-8 kb), whereas Poly(I:C) LMW has a low molecular weight (average size 0.2-1 kb). Poly(I:C) and Poly(I:C) LMW may activate the immune system differently. dsRNA is known to induce interferons (IFN) and other cytokines production. IFN induction is mediated by two different pathways. The first pathway leading to NF-kB activation depends on the dsRNA-responsive protein kinase (PKR), whereas the second path is PKR-independent and involves TLR3.

- **E. coli K12 LPS** - TLR4 agonist
  Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. LPS recognition is mediated by TLR4 which forms a complex with MD2 and CD14 leading the production of proinflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes via the adaptor TRIF.

### PRODUCT INFORMATION

**Content:**
- TLR1/2 agonist - Pam3CSK4 x HCl (10 µg)
- TLR2 agonist - HKLM (106 cells)
- TLR3 agonist - Poly(I:C) (500 µg)
- TLR3 agonist - Poly(I:C) LMW (500 µg)
- TLR4 agonist - *E. coli K12* LPS (100 µg)
- TLR5 agonist - *S. typhimurium* Flagellin (10 µg)
- TLR6/2 agonist - FSL-1 (10 µg)
- TLR7 agonist - Imiquimod (25 µg)
- TLR8 agonist - ssRNA40 (25 µg)
- TLR9 agonist - ODN2006 (100 µg - 12.98 nmol)
- 2x 2 ml endotoxin-free water

**Storage and stability:**

<table>
<thead>
<tr>
<th>TLR Ligands</th>
<th>Lyophilized</th>
<th>Resuspended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pam3CSK4</td>
<td>1 year @ 4°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
<tr>
<td>HKLM</td>
<td>1 year @ 4°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
<tr>
<td>Poly(I:C)</td>
<td>1 year @ 4°C</td>
<td>1 month @ 4°C, 1 year @ -20°C</td>
</tr>
<tr>
<td>Poly(I:C) LMW</td>
<td>1 year @ 4°C</td>
<td>1 month @ 4°C, 1 year @ -20°C</td>
</tr>
<tr>
<td>LPS-EK</td>
<td>1 year @ -20°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
<tr>
<td>SF-FLA</td>
<td>1 year @ -20°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
<tr>
<td>FSL-1</td>
<td>1 year @ -20°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
<tr>
<td>Imiquimod</td>
<td>1 year @ -20°C</td>
<td>6 months @ -20°C</td>
</tr>
<tr>
<td>ssRNA40</td>
<td>1 year @ -20°C</td>
<td>1 week @ 4°C</td>
</tr>
<tr>
<td>ODN2006</td>
<td>1 year @ -20°C</td>
<td>1 month @ 4°C, 6 months @ -20°C</td>
</tr>
</tbody>
</table>

- Products are shipped at room temperature.

### DESCRIPTION

- **Pam3CSK4 - TLR1/2 agonist**
  Pam3CSK4 is a synthetic tripalmitoylated lipopeptide that mimics the acylated amino terminus of bacterial lipoproteins. Pam3CysSerLys4 (Pam3CSK4) is a potent activator of the proinflammatory transcription factor NF-kB. Recognition of Pam3CSK4 is mediated by TLR2 which cooperates with TLR1 through their cytoplasmic domain to induce the signaling cascade leading to the activation of NF-kB.

  **Molecular weight:** 1509.6 - 109.5

- **HKLM - TLR2 agonist**
  HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces a strong nonspecific response characterized by the secretion of proinflammatory cytokines. This response is mediated by TLR2. Stimulation with HKLM induces immediate activation of NF-kB and the production of proinflammatory cytokines.

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**For research use only**
Version # 11B03-MM
**Imiquimod - TLR7 agonist**

Imiquimod (R837), an imidazoquinoline amine analogue to guanosine, is an immune response modifier with potent indirect antiviral activity. Imiquimod is an approved treatment for external genital warts caused by human papillomavirus infection. This low molecular synthetic molecule induces the production of cytokines such as IFN-α. Unlike R848, Imiquimod activates only TLR7 but not TLR8. This activation is MyD88-dependent and leads to the induction of the transcription factor NF-κB.

Molecular weight: 240.3

**ssRNA40 - TLR8 agonist**

ssRNA40 is a 20-mer phosphothioate protected single-stranded RNA oligonucleotide containing a GU-rich sequence. ssRNA40 is complexed with the cationic lipid LyoVec® to protect it from degradation and facilitate its uptake, and when complexed to unmethylated CpG dinucleotides in particular sequence contexts, it activates plasmacytoid dendritic cells (pDC) to produce IFN-α. ssRNA40 is complexed with the cationic lipid LyoVec® (ratio 1:2), to protect it from degradation and facilitate its uptake, and when complexed to unmethylated CpG dinucleotides in particular sequence contexts, it activates plasmacytoid dendritic cells (pDC) to produce IFN-α. ssRNA40 complexes are recognized by TLR8 in humans and TLR7 in mice.

5'-GCCCGUCUGUUGUGACUC-3' (phosphorothioate bases)

**ODN2006 (type B) - TLR9 agonist**

CpG ODNs are synthetic oligonucleotides containing unmethylated CpG dinucleotides in particular sequence contexts that induce strong immunostimulatory effects through the activation of TLR9. Two types of CpG ODNs have been described. Type A (or D) ODNs preferentially activate plasmacytoid dendritic cells (pDC) to produce IFN-α, whereas type B (or K) ODNs induce the proliferation of B cells and the secretion of IgM and IL-6.

5' - tcg tcg ttt cgt cgt ttt gtc gtt -3' (phosphorothioate bases)

**METHODS**

**Preparation of TLR agonist stock solutions**

<table>
<thead>
<tr>
<th>Product</th>
<th>Working concentration</th>
<th>Stock solution Concentration</th>
<th>Volume of solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pam3CSK4</td>
<td>0.1-1 µg/ml</td>
<td>100 µg/ml</td>
<td>100 µl H2O</td>
</tr>
<tr>
<td>HKLM</td>
<td>10⁶ cells/ml</td>
<td>10⁶ cells/ml</td>
<td>100 µl H2O</td>
</tr>
<tr>
<td>Poly(I:C)</td>
<td>10 ng-10 µg/ml</td>
<td>1 µg/ml</td>
<td>500 µl H2O</td>
</tr>
<tr>
<td>Poly(I:C) LMW</td>
<td>30ng-10 µg/ml</td>
<td>1 mg/ml</td>
<td>500 µl H2O</td>
</tr>
<tr>
<td>LPS</td>
<td>10 ng-10 µg/ml</td>
<td>1 µg/ml</td>
<td>1 ml H2O</td>
</tr>
<tr>
<td>Flagellin</td>
<td>10 ng-10 µg/ml</td>
<td>100 µg/ml</td>
<td>100 µl H2O</td>
</tr>
<tr>
<td>FSL-1</td>
<td>1 ng-1 µg/ml</td>
<td>100 µg/ml</td>
<td>100 µl H2O</td>
</tr>
<tr>
<td>Imiquimod</td>
<td>0.25-10 µg/ml</td>
<td>100 µg/ml</td>
<td>250 µl H2O</td>
</tr>
<tr>
<td>ssRNA40</td>
<td>0.25-10 µg/ml</td>
<td>100 µg/ml</td>
<td>250 µl H2O</td>
</tr>
<tr>
<td>ODN2006</td>
<td>5 µM</td>
<td>500 µM</td>
<td>26 µl H2O</td>
</tr>
</tbody>
</table>

**TLR stimulation**

- Transfect your cell line with an NF-κB-inducible reporter plasmid, i.e. a plasmid carrying a reporter gene, such as SEAP or luciferase, under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter.

**References**