LPS-EB Biotin

Biotinylated ultrapure lipopolysaccharide from *E. coli* 0111:B4 strain; TLR4 ligand

Catalog code: tlrl-3blps

https://www.invivogen.com/lps-eb-biotin

For research use only

Version 18K14-MM

PRODUCT INFORMATION

Contents
- 500 µg biotinylated ultrapure *E. coli* O111:B4 LPS (LPS-EB Biotin)
- 1.5 ml endotoxin-free water

Storage and stability
- LPS-EB Biotin is shipped at room temperature. Upon receipt, it should be stored at -20°C.
- Upon resuspension, prepare aliquots and store at 4°C or -20°C.
- Resuspended product is stable for 1 month at 4°C and for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control
- Endotoxin levels: ≥ 1 x 10⁵ EU/mg (measurement with HEK-Blue™ LPS Detection Kit 2).
- The TLR4 activity has been confirmed using HEK-Blue™ TLR4 cells.
- The presence of other bacterial components (e.g. lipopolysaccharides) has been tested for in HEK-Blue™ TLR2 cells.
- Biotin coupling has been validated by flow cytometry.

DESCRIPTION

LPS-EB Biotin is biotinylated ultrapure lipopolysaccharide (LPS) from the Gram negative bacteria *E. coli* 0111:B4. LPS is recognized by toll-like receptor 4 (TLR4) which interacts with three different extracellular proteins: LPS binding protein (LBP), CD14, and, myeloid differentiation protein 2 (MD-2), to induce a signaling cascade leading to the activation of NF-κB and the production of proinflammatory cytokines. Most LPS preparations on the market are contaminated by other bacterial components, such as lipoproteins, thus activating TLR2 signaling as well as TLR4 signaling. The ultrapure preparation that InvivoGen provides only activates the TLR4 pathway. In this product, biotin has been bound to LPS’s oxidized-carbohydrates using biotin-LC-hydrazide as previously described. The labeled ligand retains the biological activity of LPS. LPS-EB Biotin can be used for the detection of LPS binding sites by cytometry or fluorescence microscopy.

PRODUCT PROPERTIES

Source: *Escherichia coli* 0111:B4

Specificity: TLR4

Working concentration: 10 ng-10 µg/ml

Solubility: 1 mg/ml in water

METHODS

Preparation of stock solution (1 mg/ml)
- Add 500 µl endotoxin-free water (provided) and homogenize by vortexing.

Several applications of LPS-EB Biotin have been reported. The procedure below describes the use of LPS-EB Biotin for the detection of LPS binding sites by fluorescence microscopy or cytometry with RAW-Blue™ cells which express TLR4. Depending on the cell type studied, the procedure may need to be modified. Other applications have not been tested by InvivoGen.

Staining procedure

**Day 1**
1. Prepare a suspension of RAW-Blue™ cells at 5 x 10⁵ cells/ml in RPMI with 10% heat-inactivated fetal calf serum.
2. Add 450 µl of the cell suspension into each well of a 24-well plate.
3. Dispense 50 µl of LPS-EB Biotin (final concentration 10 ng-10 µg/ml) per well.
4. Incubate plate overnight at 37°C.

**Day 2**
1. Detach cells using a cell scraper and transfer cells to a 5 ml tube.
2. Fill the tube with cold PBS and centrifuge for 5 minutes at 1200 rpm.
3. Resuspend the pellet in 100 µl PBS containing FeR blocking agent.
4. Fix and permeabilize cells
5. Incubate cells with a fluorescently labeled anti-biotin for 30 min at 4°C.
6. Wash the cells with PBS.
7. Observe stained cells by fluorescence microscopy or cytometry.

RELATED PRODUCTS

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<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEK-Blue™ hTLR4 Cells (human TLR4)</td>
<td>hkb-htlr4</td>
</tr>
<tr>
<td>HEK-Blue™ LPS Detection Kit 2</td>
<td>rep-lps2</td>
</tr>
<tr>
<td>LPS-EB Ultrapure (LPS from <em>E. coli</em> 0111:B4)</td>
<td>tlrl-3pelps</td>
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<tr>
<td>LPS-EB VacciGrade™</td>
<td>vac-3pelps</td>
</tr>
<tr>
<td>RAW-Blue™ Cells (mouse TLR4)</td>
<td>raw-sp</td>
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TECHNICAL SUPPORT

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