

LPS-EB Biotin

Biotinylated ultrapure lipopolysaccharide from *E. coli* O111:B4 strain; TLR4 ligand

Catalog code: tlrl-lpsbiot

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

For research use only

Version 23F20-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: $\geq 1 \times 10^5$ 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. lipoproteins) 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* O111: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- κ B and the production of proinflammatory cytokines^{1,2}.

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 described³. 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.

1. Poltorak A., 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in TLR4 gene. *Science*, 282:2085-8. 2. Latz E. et al., 2002. Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. *J Biol Chem* 277:47834. 3. Luk JM. et al., 1995. Biotinylated lipopolysaccharide binds to endotoxin receptor in endothelial and monocytic cells. *Anal Biochem* 232:217..

PRODUCT PROPERTIES

Source: *Escherichia coli* O111: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.

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, murine macrophages 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 5×10^5 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 FcR 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

Product	Description	Cat. Code
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtlr4
HEK-Blue™ LPS Detection Kit 2	LPS Detection Kit	rep-lps2
LPS-EB Ultrapure	LPS from <i>E. coli</i> O111:B4	tlrl-3pelps
LPS-EB VacciGrade™	LPS from <i>E. coli</i> O111:B4	vac-3pelps
RAW-Blue™ Cells	Mouse macrophages	raw-sp

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com