PRODUCT INFORMATION

Content:
- 5 x 10^6 EU of ultra pure LPS from *E. coli* 0111:B4 (LPS-EB Ultrapure)
- 1.5 ml endotoxin-free water

Storage:
- LPS-EB Ultrapure is shipped at room temperature and should be stored at -20°C. Lyophilized product is stable 1 year when properly stored.
- Upon resuspension, prepare aliquots of LPS-EB Ultrapure and store at 4°C for short term storage or -20°C for long term storage. Resuspended product is stable 1 month at 4°C and 6 months at -20°C. Avoid repeated freeze-thaw cycles.

DESCRIPTION

Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. Large quantities of LPS induce the overproduction of cytokines causing septic shock while suboptimal doses of LPS induce tolerance to subsequent exposure to LPS. LPS recognition is predominantly mediated by TLR4. This recognition involves the binding of LPS with lipopolysaccharide-binding protein (LBP) and subsequently with CD14 which physically associates with a complex including TLR4 and MD2. Formation of the TLR4-centered LPS receptor complex induces 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. Furthermore, the shape of Lipid A, the component responsible for the immunostimulatory activity of LPS, has been shown to direct the interaction of LPS with TLRs.

Most LPS preparations on the market are contaminated by other bacterial components, such as lipoproteins, thus activating TLR2 signaling as well as TLR4 signaling. Ultra-Pure LPS-EB was extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol. The Ultra-Pure LPS-EB preparation provided by InvivoGen only activates the TLR4 pathway.

METHODS

Preparation of a stock solution (5 x 10^6 EU/ml)
- Add 1 ml of endotoxin-free water (provided).
- Vortex until complete solubilization.
- Store at 4°C for short term storage or -20°C for long term storage.

Note: 5 x 10^6 EU/ml corresponds to 5 mg/ml.

TLR4 stimulation
Stimulation of TLR4 with LPS-EB Ultrapure can be achieved with concentrations ranging from 10^1 to 10^4 EU/ml.
- Transfect your cell line with a pNiFty plasmid, an NF-κB 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.

If your cell line does not naturally express TLR4, MD2 and CD14, cotransfect with a TLR4 expression plasmid such as pUNO1-TLR4 and an MD2/CD14 expression plasmid such as pDUO2-hMD2/CD14.

Note: Alternatively, evaluate TLR4 activation using reporter cells, such as InvivoGen’s HEK-Blue™ hTLR4 cells which express the human TLR4, MD2, CD14 and SEAP reporter genes. NF-kB production in these cells can be easily quantified using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection.

- Twenty-four to forty-eight hours after transfection, stimulate cells with 10^1 to 10^4 EU/ml LPS-EB Ultrapure for 6 to 24 hours.
- Determine LPS stimulation on TLR4 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

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