

HEK-Blue™ LPS Detection Kit

Detection Method based on the activation of Toll-Like Receptor 4

For the detection of lipopolysaccharide from gram-negative bacteria in biological reagents

Catalog # rep-lps

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This package insert must be read in its entirety before using this product

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INTRODUCTION

Lipopolysaccharide (LPS), the major cell wall component of gram-negative bacteria, induces the activation of NF-κB and the production of proinflammatory cytokines¹.

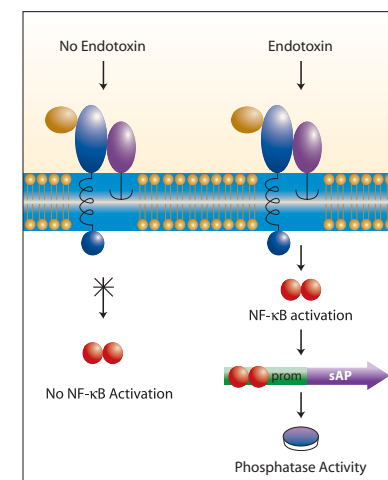
In vivo, this response can cause fever, septic shock and eventually death of the animal².

In vitro, it can introduce a bias in experiments involving cells sensitive to low levels of LPS such as monocytes. In addition, repeated passages of cell lines in a medium containing LPS might render these cells unresponsive to further stimulation by LPS. This desensitization of the cells, termed LPS tolerance³, does not only affect inflammatory responses but also other essential functions including antigen presentation by monocytes⁴. Thus, monitoring the presence of LPS in biological reagents is crucial. InvivoGen provides the HEK-Blue™ LPS Detection Kit, a simple, rapid and reliable system to detect the presence of lipopolysaccharides in your samples.

KIT DESCRIPTION

The HEK-Blue™ LPS Detection kit is based on the ability of TLR4 to recognize structurally different LPS from gram-negative bacteria and in particular lipid A, their toxic moiety. Proprietary cells engineered to become extremely sensitive to LPS, called HEK-Blue™-4 cells, are the main feature of the HEK-Blue™ LPS detection kit. The presence of very low concentrations of LPS, starting as low as 0.3 ng/ml, is detected by the HEK-Blue™-4 cells leading to the activation of NF-κB. Using HEK-Blue™ Detection, a specific detection medium, NF-κB activation can be observed with the naked eye or quantified spectrophotometrically.

This simple detection test requires only basic cell culture knowledge and may be easily established as a routine procedure in the lab.



HEK-Blue™-4 Cells

HEK-Blue™-4 cells are engineered HEK293 cells stably transfected with multiple genes involved in TLR4 recognition that include TLR4 and the co-receptors MD2 and CD14 (see "Specificity and Sensitivity", pages 15-16). In addition, HEK-Blue™-4 cells stably express an optimized alkaline phosphatase gene engineered to be secreted (sAP), placed under the control of a promoter inducible by several transcription factors such as NF-κB and AP-1.

This reporter gene allows the monitoring of the signaling through TLR4, based predominantly on the activation of NF-κB which reflects the presence of LPS in the sample to be tested. The phosphatase activity is detected by the use of HEK-Blue™ Detection medium.

HEK-Blue™ Selection Mix

HEK-Blue™ Selection is a solution that combines several selective antibiotics. These antibiotics guarantee the persistent expression of the various transgenes introduced in HEK-Blue™-4 cells. Furthermore, Normocin™ is included in the kit to protect HEK-Blue™-4 cells from any potential microbial contamination, whether caused by mycoplasma, bacteria or fungi.

HEK-Blue™ Detection

HEK-Blue™ Detection is a medium specifically designed for the detection of NF-κB activation in HEK-Blue™ cells. This medium turns into a blue color in the presence of phosphatase activity. The product of the reaction with the substrate is cytotoxic and results in the death of the cells.

HEK-Blue™ Detection is a powdered medium provided in individually sealed pouches. Each pouch allows the preparation of 50 ml of detection medium.

E. coli K12 LPS

The sensitivity of the kit can be assessed by using serial dilutions of *E. coli* K12 LPS prepared in endotoxin-free water. A positive response should be obtained for a final concentration ≥ 0.3 ng/ml.

PROCEDURES SUMMARY

Handling procedure of HEK-Blue™-4 cells

1. Thaw HEK-Blue™-4 cells
2. Expand HEK-Blue™-4 cells in the presence of HEK-Blue™ Selection
3. Make your frozen stock of HEK-Blue™-4 cells

LPS detection procedure

1. Grow HEK-Blue™-4 cells up to 60-80% confluence in growth medium supplemented with HEK-Blue™ Selection
2. Prepared reagents following instructions (HEK-Blue™ Detection medium, diluted Trypsin-EDTA solution, positive controls).
3. Warm up samples and controls at 37°C. Mix vigorously by vortexing.
Add 20 μ l of sample or control per well of a 96-well plate.
4. Harvest cells and resuspend gently in HEK-Blue™ Detection medium.
5. Add 200 μ l (2.5×10^4 cells) of the cell suspension to each well.
6. Incubate the plate(s) 18-24H at 37°C in 5% CO₂.
7. Assess the blue color by the naked eye or the OD at 620-655 nm.

Purple or blue color = Presence of LPS	Pink color = No LPS
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KIT INFORMATION

Contents

The HEK-Blue™ LPS Detection Kit contains the following components:

- 1 vial of HEK-Blue™ cells (3-5x 10⁶ cells)
- 4 tubes of 250X HEK-Blue™ Selection (2 ml)
- 4 tubes of 500X Normocin™ (1 ml for 500 ml of culture medium)
- 2 pouches of HEK-Blue™ Detection
- 1 tube of *E. coli* K12 LPS (100 µg) as a positive control
- 1 tube of endotoxin-free water (1.5 ml) as a negative control

Note: Most components of the HEK-Blue™ LPS Detection kit can be purchased separately (see “Related Products” page 19).

Storage and stability

- The HEK-Blue™ LPS Detection Kit is shipped on dry ice.
- Upon receipt HEK-Blue™ cells must be thawed **immediately** and grown according to handling procedures described on page 9.
- Store unopened HEK-Blue™-4 Selection, Normocin™ and *E. coli* K12 LPS at -20°C for up to 12 months.
- Store unopened HEK-Blue™ Detection and Endotoxin-free water at room temperature for up to 6 months.
- Resuspended and filtered HEK-Blue™ Detection is stable 2 weeks at 4°C and at least 2 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

ADDITIONAL MATERIALS REQUIRED

Reagents required

- Dulbecco’s modified Eagle’s medium (DMEM), high glucose (4.5 g/L)

Note: If using DMEM without glutamine, add 2 mM glutamine.

- Penicillin-Streptomycin solution
- Fetal Bovine Serum (FBS) without endotoxin

Note: For better results, we recommend using FBS from Hyclone (#SH30071-03) or Cambrex (#DE14-801F).

- Trypsin-EDTA (0.05% Trypsin, EDTA.4Na)
- Endotoxin-free water

Note: Most commercial spring waters are endotoxin-free.

- Phosphate buffered saline (PBS)
- Dimethylsulfoxide (DMSO)

Supplies required

- Laminar flow hood
- Centrifuge
- Water bath (37°C)
- Inverted microscope
- CO2 incubator
- Sterile cell culture plasticware: tubes, pipettes, 25 cm² and 175 cm² flasks, flat-bottom 96-well plates, tips.
- Cryotubes
- 250 ml sterile bottles
- 0.2 µm filters
- Counting cell (e.g. Malassez)

Optional:

- Multichannel pipettes (200 µl or 300 µl) and autoclavable reagent reservoirs
- Freezing container
- Microplate reader with 655 nm filter

SAFETY CONSIDERATION

The HEK-Blue™ LPS Detection Kit contains antibiotics and products of biological and bacterial origins that must be handled observing the usual safety precautions (wear appropriate protective equipment, do not ingest, do not inhale).

HEK-Blue™-4 cells require **Biosafety Level 2**.

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. This cell line is known to contain an agent associated with human disease. This cell line is sent with the condition that you are responsible for its safe storage, handling and use. InvivoGen is not liable for damages or injuries resulting from receipt and/or use of an InvivoGen culture. Detailed discussions of laboratory safety procedures are provided in Laboratory Safety: Principles and Practices (Fleming et al., 1995), the ATCC manual on quality control (Hay et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and the U.S. Government Publication, Biosafety in Microbiological and Biomedical Laboratories, 4th ed. HHS publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Note: InvivoGen highly recommends that protective gloves and clothing always be used and a full mask always be worn when handling frozen vials.

