HKEB

Heat Killed *E.coli 0111:B4* - TLR2 & TLR4 agonist

Catalog # tlrl-hkeb2

For research use only

Version # 12F07-MM

PRODUCT INFORMATION

Content:

- 10^{10} freeze-dried cells of Heat Killed *E.coli 0111:B4* (HKEB)
- 1.5 ml sterile endotoxin-free water

Endotoxin level: >1 EU/10^9 cells

Storage:

- HKEB is provided lyophilized and shipped at room temperature. Store at 4˚C.
- Upon resuspension, prepare aliquots of HKEB and store at 4°C for short term storage or -20˚C for long storage.
- Resuspended product is stable 1 month at 4°C and at least 1 year at -20°C when properly stored.

DESCRIPTION

HKEB is a heat killed preparation of the gram negative bacterium, *E.coli O111:B4*. Cell wall components from this bacterium, such as peptidoglycan (PGN) and lipopolysaccharide (LPS), are recognized by the toll-like receptor (TLR) 2 and TLR4[1]. It has been demonstrated that HKEB can stimulate TLR2 and induce the production of NF-κB and pro-inflammatory cytokines, such as IL-8[2]. HKEB is a potent stimulator of TLR2 and TLR4.

1. Takeuchi O. *et al*., 1999. Differential roles of TLR2 and TLR4 in recognition of gram negative and gram-positive bacterial cell wall components. Immunity, 11(4):443-51.
2. van Riet E. *et al*., 2009. Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for TH1/TH2 polarization. BMC Immunology, 10:9.

METHODS

Preparation of stock solution (10^{10} HKEB cells/ml)

Stimulation of TLR2 and TLR4 can be achieved with 10^5 - 10^7 HKEB cells/ml.

- Add 1 ml sterile endotoxin-free water (provided) to rehydrate the pellet.
- Vortex for 10 seconds or until homogenized.

Note: Rehydrated HKEB results in a milky suspension.

Example TLR2 and TLR4 stimulation using HKEB

HKEB can be used to stimulate TLR2 or TLR4 in HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells, respectively. HEK-Blue™-TLR cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress a TLR gene. For more information visit: www.invivogen.com/hek-blue-tlr-cells

- Add 10^5 - 10^7 HKEB cells/ml to HEK-Blue™ TLR cells (prepare cell suspension according to data sheet).
- Incubate cells and HKEB for 6 - 24 h at 37°C, 5% CO_2.
- Determine TLR2 or TLR4 stimulation with HKEB by assessing cytokine expression using an ELISA, or SEAP expression using a SEAP detection medium, such as QUANTI-Blue™.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 cells	hkb-htlr2
HEK-Blue™ hTLR4 cells	hkb-htlr4
QUANTI-Blue™	rep-qb1
Other TLR2 ligands:	
FSL-1 (synthetic diacylated lipoprotein)	tlrl-fsl
HKLM (heat killed *L.monocytogenes*)	tlrl-hklm
LM-MS (Lipomannan from *M.smegmatis*)	tlrl-lmm2
Pam3CSK4 (synthetic triacylated lipoprotein)	tlrl-pms

TECHNICAL SUPPORT
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