HKLM

Heat Killed *Listeria monocytogenes* - TLR2 ligand

Catalog # tlrl-hklm

For research use only

Version # 11C02-MM

PRODUCT INFORMATION

Content:

- 10^{10} freeze-dried cells of Heat Killed *Listeria Monocytogenes* (HKLM)
- 1.5 ml endotoxin-free water

Storage :

- HKLM is shipped at room temperature and should be stored at 4°C.
- Upon resuspension, prepare aliquots of HKLM and store at 4°C for short term storage or -20°C for long storage.
- Product is stable 1 month at 4°C and 6 months at -20°C when properly stored.

DESCRIPTION

HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces a strong nonspecific response characterized by the secretion of proinflammatory cytokines. This response is mediated by the interaction between MyD88 and several TLRs, mainly TLR2[1], a Toll-like receptor involved in the recognition of multiple products of Gram+ bacteria. Stimulation with HKLM induces immediate activation of NF-κB and the production of proinflammatory cytokines[2,3].

1. Flo TH. *et al.*, 2000. Human toll-like receptor 2 mediates monocyte activation by Listeria monocytogenes, but not by group B streptococci or lipopolysaccharide. J Immunol, 164(4):2064-9. **2. Hauf N. *et al.*, 1997.** Listeria monocytogenes infection of P388D1 macrophages results in a biphasic NF-kB (RelA/p50) activation induced by lipoteichoic acid and phospholipases and mediated by IκBα and IκBβ degradation. Proc. Natl. Acad. Sci. USA 94(17):9394-9. **3. Plevy SE. *et al.*, 1998.** Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional synergy between C/EBP and Rel proteins. Mol Cell Biol. 17(8):4572-88. **4. Schindler U. & Baichwal VR., 1994.** Three NF-kB binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol, 14(9):5820-5831.

METHODS

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

Stimulation of TLR2 can be achieved with 10^7 - 10^8 HKLM/ml.

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

Note: Resuspended HKLM results in a milky solution.

HKLM stimulation

- Transfect your cell line with 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[4].

Note: InvivoGen provides pNiFty, a family of NF-κB-inducible reporter plasmids that can be transfected transiently (pNiFty) or stably (pNiFty2). pNiFty plasmids are available either with the SEAP or luciferase reporter genes (see Related Products).

If your cell line does not naturally express TLR2, cotransfect with a TLR2 expressing plasmid, such as pUNO-TLR2.

Note: Alternatively, evaluate TLR2 activation using reporter cells, such as InvivoGen's HEK-Blue™ hTLR2 cells which express the human TLR2 and SEAP reporter genes. NF-κB 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 10^7 - 10^8 HKLM/ml for 6 to 24 hours.
- Determine HKLM stimulation on TLR2 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 cells	hkb-htlr2
pNiFty-Luc (Amp^R)	pnifty-luc
pNiFty-SEAP (Amp^R)	pnifty-seap
pNiFty2-Luc (Zeo^R)	pnifty2-luc
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pUNO1-hTLR2 (human gene)	puno1-htlr2
pUNO-mTLR2 (mouse gene)	puno-mtlr2

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