

# HKMT

## Heat-killed *Mycobacterium tuberculosis*; Mincle and TLR2 ligand

Catalog code: tlr1-hkmt-1, tlr1-hkmt-5

<https://www.invivogen.com/hkmt>

### For research use only

Version 24G15-AK

## PRODUCT INFORMATION

### Contents

- Heat-killed preparation of *Mycobacterium tuberculosis* H37Ra (HKMT) is provided lyophilized and is available in two quantities:
  - 10 mg; tlr1-hkmt-1
  - 5 x 10 mg; tlr1-hkmt-5
- endotoxin-free water; 1.5 ml with tlr1-hkmt and 10 ml with tlr1-hkmt-5.

### Storage and stability

- HKMT is shipped at room temperature. Upon receipt, store at 2-8°C.
- Upon resuspension, store at 2-8°C. Resuspended HKMT can be stored at 2-8°C for 6 months.

### Quality control

- The biological activity has been validated using HEK-Blue™ Mincle and HEK-Blue™ TLR2 cells.
- The absence of endotoxins has been confirmed using HEK-Blue™ TLR4 cells.

## DESCRIPTION

HKMT is a heat-killed preparation of the avirulent strain *Mycobacterium tuberculosis* H37Ra, which was derived from the virulent strain H37. The attenuated *M. tuberculosis* strain H37Ra is one of the most commonly used controls for *M. tuberculosis*.

HKMT binds the C-Type lectin, Mincle (macrophage-inducible C-type lectin)<sup>1</sup>. Upon agonist recognition, Mincle interacts with the Fc receptor common  $\gamma$ -chain (FcR $\gamma$ ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF- $\kappa$ B activation<sup>2,3</sup>.

Due to the presence of bacterial cell wall components, HKMT is recognized by TLR2 triggering NF- $\kappa$ B activation with the subsequent production of proinflammatory cytokines, such as TNF- $\alpha$ <sup>4</sup>. HKMT does not activate TLR4 even at high concentrations (upto 100  $\mu$ g/ml).

1. **Ishikawa, E. et al., 2009.** Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J. Exp. Med.* 206, 2879–2888.
2. **Schoenen H. et al., 2010.** Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. *J Immunol.* 184(6):2756-60.
3. **Kerscher B. et al., 2013.** The Dectin-2 family of C-type lectin-like receptors: an update. *Int Immunol.* 25(5):271-7.
4. **Underhill DM. et al., 1999.** Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *PNAS.* 96(25):14459-63.

## METHODS

### Preparation of stock suspension (10 mg/ml)

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

*Note: Resuspended HKMT results in a cloudy suspension. It is recommended to sonicate the solution for 5 to 10 minutes to homogenize it.*

### Working concentrations:

- 10-100  $\mu$ g/ml when used as a Mincle ligand
- 100 ng-10  $\mu$ g/ml when used as a TLR2 ligand

### HKMT-induced Mincle & TLR2 activation

HKMT can be used to stimulate Mincle in HEK-Blue™ Mincle cells. These cells were transfected with the Mincle gene and other genes from the Mincle signaling pathway. They also stably express an NF- $\kappa$ B-inducible secreted embryonic alkaline phosphatase (SEAP). For more information visit: <https://www.invivogen.com/hek-blue-clr>.

Alternatively, HKMT can be used to stimulate TLR2 in HEK-Blue™ TLR2 cells. These cells stably overexpress the TLR2 gene and an NF- $\kappa$ B-inducible secreted embryonic alkaline phosphatase (SEAP). For more information visit: <https://www.invivogen.com/hek-blue-trl2>.

1. Add 20  $\mu$ l of HKMT at various concentrations (suggested dose-response range 0.1-100  $\mu$ g/ml) in a well of a 96-well plate.
2. Add 180  $\mu$ l of cell suspension (prepare cell suspension according to data sheet) per well.
3. Incubate the plate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
4. Determine Mincle or TLR2 stimulation with HKMT by assessing SEAP expression using a SEAP detection medium, such as QUANTI-Blue™ Solution or HEK-Blue™ Detection.

## RELATED PRODUCTS

Product	Description	Cat. Code
FSL-1	TLR2 ligand	tlrl-fsl
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
HEK-Blue™ hTLR2 Cells	Human TLR2 reporter cells	hkb-htlr2
HEK-Blue™ mTLR2 Cells	Murine TLR2 reporter cells	hkb-mtlr2
HEK-Blue™ mMincle Cells	Murine Mincle reporter cells	hkb-mmcl
Pam <sub>3</sub> CSK <sub>4</sub>	TLR2 ligand	tlrl-pms
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
TDB	Mincle Ligand	tlrl-tdb

### TECHNICAL SUPPORT

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