TDM

Trehalose-6,6-dimycolate; Mincle ligand

Catalog code: tlrl-tdm

http://www.invivogen.com/tdm

For research use only

Version # 18A10-MM

PRODUCT INFORMATION

Contents

2 x 1 mg Trehalose-6,6-dimycolate (TDM)

Required material

Isopropanol **(not provided)**

Storage

- TDM is shipped at room temperature. Store at 2-8 °C. Lyophilized product is stable for 1 year when properly stored.
- Upon resuspension, prepare aliquots and store product at -20 °C. Resuspended product is stable for 6 months when properly stored. Do **not** refreeze once thawed.

Quality control

- Chemical characterization has been performed by NMR and mass spectrometry.
- The biological activity has been tested using HEK-Blue™ Mincle cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Trehalose-6,6-dimycolate (TDM, also known as cord factor) is a unique glycolipid found in the cell wall of *Mycobacterium tuberculosis*. It is the most abundant lipid found in these bacteria and its presence is believed to correlate with the virulence of tuberculosis. Consequently, it is the most studied immunostimulatory component of *M. tuberculosis*[1]. Two C-type lectin receptors, macrophage-inducible C-type lectin (Mincle) and macrophage C-type lectin (MCL; also known as Dectin-3), are required for TDM-mediated immune responses[2]. TDM is recognized through its carbohydrate moiety by Mincle and through its lipid tail by MCL[2]. These two receptors form a heterodimer and pair with the signaling adaptor molecule Fc receptor common γ-chain (FcRγ) which in turn recruits spleen tyrosine kinase (Syk) triggering CARD9-Bcl10-MALT1 signaling. Induction of this pathway leads to NF-κB activation and production of a large number of inflammatory cytokines[3,4]. Notably, as TDM orients the maturation of T-helper (Th) cells toward Th1 and Th17 subsets[4], this product may be useful for studying Th1/Th17-polarized immune responses in cellular assays with antigen-presenting cells (APCs).

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. Richardson MB. & Williams SJK., 2014.** MCL and Mincle: C-Type Lectin Receptors That Sense Damaged Self and Pathogen-Associated Molecular Patterns. Front Immunol. 5:288; **3. Miyake Y. *et al.*, 2015.** C-Type Lectin Receptor MCL Facilitates Mincle Expression and Signaling through Complex Formation. J Immunol. 194(11):5366-74. **4. Geijtenbeek TB. & Gringhuis SI., 2016.** C-type lectin receptors in the control of T helper cell differentiation. Nat. Rev. Immunol. 16(7):433-48.

CHEMICAL PROPERTIES

Source: Isolated from *Mycobacterium tuberculosis* H37Ra
CAS number: 61512-20-7
Solubility: 500 µg/ml in isopropanol
Working concentration: 300 ng-10 µg/ml

METHODS

Preparation of stock suspension (500 µg/ml)

- Add 2 ml of isopropanol (**not provided**) to 1 mg of TDM.
- Heat at 60 °C for 2 minutes, sonicate for 20 seconds and vortex until completely dissolved.
- Use immediately or store aliquots at -20 °C. Heat frozen aliquots at 60 °C for 2 minutes before use. Do **not** refreeze product.
- Prepare dilutions with isopropanol.

Mincle activation using TDM

TDM can be used to stimulate Mincle in HEK293 cells that were transfected with the Mincle gene and other genes from the Mincle signaling pathway. These cells also stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). For more information visit: http://www.invivogen.com/hek-blue-clr

Day 1

1. Dispense 20 µl of TDM suspension at various concentrations (300 ng to 10 µg/ml final concentration) per well in a 96-well plate.
2. Ensure that the TDM suspension is evenly distributed on the surface of the well.
3. Allow to dry for 1 hour at room temperature (15-25 °C).
4. Prepare a cell suspension (~280,000 cells per ml) and add 180 µl of this suspension (~50,000 cells) to each TDM-containing well.
5. Incubate the cells for 20-24 hours at 37 °C and 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ following the instructions on the pouch.
7. Add 20 µl of supernatant to each well containing 180 µl of QUANTI-Blue™.
8. Incubate the plate at 37 °C for 30 minutes to 6 hours.
9. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ mMincle	hkb-mmcl
HKMT (heat-killed *M. tuberculosis*)	tlrl-hkmt-1
TDB (trehalose-6,6-dibehenate)	tlrl-tdb
pUNO1-hMINCLE (human gene)	puno1-hmincle
pUNO1-mMINCLE (murine gene)	puno1-mmincle

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