TDB

Trehalose-6,6-dibehenate; Mincle ligand

Catalog code: tlrl-tdb

https://www.invivogen.com/tdb

For research use only

Version 23F22-MM

PRODUCT INFORMATION

Contents

- 2 x 1 mg Trehalose-6,6-dibehenate (TDB)

Storage and stability

- TDB is provided as a powder and shipped at room temperature. Upon receipt, store lyophilized product at -20 °C.
- Resuspended product is stable for 6 months at 4 °C when properly stored.

Quality control

- Chemical characterization has been performed by NMR.
- 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-dibehenate (TDB) is a synthetic analog of trehalose-6,6-dimycolate (TDM, also known as cord factor), which is the most studied immunostimulatory component of *Mycobacterium tuberculosis*[1]. TDB binds to two C-type lectin receptors, macrophage-inducible C-type lectin (Mincle) and macrophage C-type lectin (MCL; also known as Dectin-3). TDB is recognized through its carbohydrate moiety by Mincle and through its lipid tail by MCL[2-4]. Upon TDB recognition Mincle forms a heterodimer with MCL which then interacts with the Fc receptor common γ-chain (FcRγ). Subsequent intracellular signaling through Syk-CARD9-dependent NF-κB activation leads to production of Th1/Th17 polarization cytokines and chemokines[4].

1. Holten-Andersen L. *et al.*, 2004. Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect. Immun. 72:1608-1617. **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, 2756–2760. **3. Werninghaus K. *et al.*, 2009.** Adjuvanticity of a synthetic cord factor analogue for subunit *Mycobacterium tuberculosis* vaccination requires FcRγ–Syk–Card9–dependent innate immune activation. J Exp Med. 16;206(1):89-97. **4. Geijtenbeek T.B. & Gringhuis S.I., 2016.** C-type lectin receptors in the control of T helper cell differentiation. Nat. Rev. Immunol. 16(7):433-48.

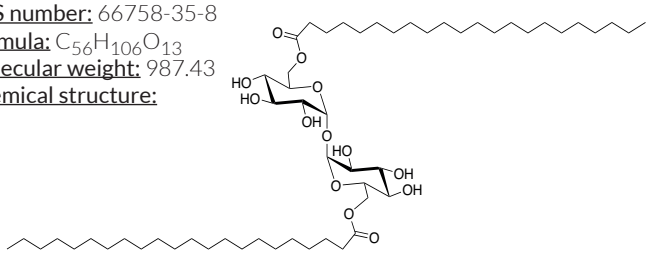
CHEMICAL PROPERTIES

CAS number: 66758-35-8

Formula: $C_{56}H_{106}O_{13}$

Molecular weight: 987.43

Chemical structure:



METHODS

Preparation of stock suspension (1 mg/ml)

- Add 100 µl DMSO to 1 mg TDB, heat at 60 °C (approx. 15-30 seconds) and vortex.
- Once resuspended, immediately add 900 µl sterile phosphate buffered saline (PBS without Ca^{2+} and Mg^{2+}), heat for 10-15 minutes at 60 °C and homogenize by vortexing for 30 seconds.

Note: *Following the addition of PBS, the suspension may appear slightly cloudy containing floating fine particles.*

- Store at 4 °C or prepare dilutions using a buffered solution for immediate use. Prior to each use, bring suspension to room temperature and vortex to homogenize.

Working concentration: 1-100 µg/ml

Mincle activation using TDB

TDB 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: https://www.invivogen.com/hek-blue-clr.

Day 1

1. Dispense 20 µl of TDB suspension at various concentrations (300 ng to 10 µg/ml final concentration) per well in a 96-well plate.
2. Ensure that the TDB 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 TDB-containing well.
5. Incubate the cells for 20-24 hours at 37 °C and 5% CO_2.

Day 2

1. Prepare QUANTI-Blue™ Solution following the instructions on the technical datasheet.
2. Add 20 µl of supernatant to each well containing 180 µl of QUANTI-Blue™ Solution.
3. Incubate the plate at 37 °C for 30 minutes to 6 hours.
4. 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
QUANTI-Blue™ Solution	rep-qbs
TDB VacciGrade™	vac-tdb

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com