TDB-HS15

Trehalose-6,6-dibehenate formulated with Kolliphor® HS 15 - Mincle ligand

Catalog # tlrl-stdb

For research use only

Version # 15A21-MM

PRODUCT INFORMATION

Content:

- 2 x 1 mg Trehalose-6,6-dibehenate (TDB) formulated with Kolliphor® HS 15

Storage:

- TDB-HS15 is provided as a powder and shipped at room temperature. Store lyophilized product at -20 °C.
- Resuspended product is stable for 6 months at 4 °C when properly stored.

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 the C-Type lectin, Mincle (macrophage-inducible C-type lectin)[1, 2]. Upon TDB recognition Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation. Syk induces also the mobilization of intracellular calcium (Ca^{2+}) and the activation of the calcineurin-NFAT pathway. It has been shown that FcRγ–Syk signaling is essential for TDB-induced activation of antigen-presenting cells (APCs)[2, 3]. In order to produce a homogenous suspension, TDB was formulated using Kolliphor® HS 15 (CAS no. 70142-34-6), a low toxicity non-ionic surfactant[4].

Kolliphor® HS 15 (former tradename Solutol® HS 15) is a registered trademark of BASF.

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, 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. BASF Technical Leaflet MEF 151e, 1986.** Solutol* HS 15 polyethyleneglycol 660 hydroxystearate as nonionic solubilizer for injection solutions.

CHEMICAL PROPERTIES OF TDB

CAS number: 66758-35-8

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

Molecular weight: 987.43

METHODS

Preparation of stock suspension (1 mg/ml):

- Add 100 µl DMSO to 1 mg TDB-HS15, 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 homogenize by vortexing for 30 seconds.

Working concentration: 0.3 - 100 µg/ml

Induction of Mincle using TDB-HS15:

TDB can be used to stimulate cells expressing Mincle, such as macrophages. The induction of Mincle can be easily studied in InvivoGen's RAW-Blue™ cells, murine macrophages stably expressing an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). A protocol for the induction of Mincle in RAW-Blue™ cells is given below:

- Add 20 µl of TDB-HS15 at various concentrations (0.3 - 100 µg/ml) in a well of a 96-well plate.
- Add 180 µl of RAW-Blue™ cell suspension (~100,000 cells) per well.
- Incubate the plate for 20 - 24 h at 37 °C, 5% CO_2.
- Collect 50 µl of supernatant and add to a well of a 96-well plate containing 150 µl of QUANTI-Blue™, a SEAP detection medium.
- Incubate the plate at 37 °C for 1 - 3 h.
- Determine SEAP levels using a spectrophotometer at 620 - 655 nm.

RELATED PRODUCTS

Product	Catalog Code
pUNO1-hMINCLE (human gene)	puno1-hmincle
pUNO1-mMINCLE (mouse gene)	puno1-mmincle
QUANTI-Blue™	rep-qb1
RAW-Blue™ Cells	raw-sp

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