

β -GlcCer

C24:1 Glucosyl(β) Ceramide; Mincle ligand

Catalog code: tlr1-bglcer

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

For research use only

Version 19H05-MM

PRODUCT INFORMATION

Contents

5 mg C24:1 Glucosyl(β) Ceramide (β -GlcCer)

Storage and stability

- β -GlcCer is provided as a solid and shipped at room temperature. Upon receipt, store product at -20°C .

- Store resuspended product in an upright position at -20°C . Resuspended product is stable for 6 months when properly stored.

Note: To avoid possible leakage or evaporation, we recommend to wrap plastic film around the lid of the vial containing the resuspended product.

Quality control

- Purity: $\geq 95\%$ (UHPLC)

- The biological activity has been tested using cellular assays.

- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Beta-glucosylceramide (β -GlcCer) is a naturally occurring glycolipid that specifically activates the macrophage-inducible C-type lectin (Mincle) receptor¹. Mincle is a member of the Dectin-2 family, that recognizes a variety of exogenous and endogenous stimuli, such as mycobacteria and necrotic cells. β -GlcCer is an endogenous metabolite which is recognized as a damage-associated molecular pattern (DAMP) and associated with cell death^{1,2}. In healthy individuals, β -GlcCer is located in the endoplasmic reticulum/Golgi apparatus. Following cell damage, it is released into the extracellular milieu where it acts as a signal of cell damage. Of note, the accumulation of β -GlcCer within cells is believed to cause systemic inflammation in Gaucher disease¹. Using InvivoGen's HEK-Blue™ Mincle reporter cells, β -GlcCer was established as an endogenous Mincle agonist¹. Upon β -GlcCer recognition, Mincle interacts with the Fc receptor common γ -chain (FcR γ) triggering Syk-dependent signaling resulting in NF- κ B, NFAT, and AP-1 activation. Due to its pro-inflammatory activity, β -GlcCer has been identified as a potential adjuvant for vaccination against infectious diseases and cancer¹.

1. Nagata M. *et al.*, 2017. Intracellular metabolite β -glucosylceramide is an endogenous Mincle ligand possessing immunostimulatory activity. PNAS. 114(16): E3285-E3294. 2. Ishikawa A. *et al.*, 2019. Essential roles of C-type lectin Mincle in induction of neuropathic pain in mice. Sci Rep. 9(1):872.

CHEMICAL PROPERTIES

CAS number: 887907-50-8

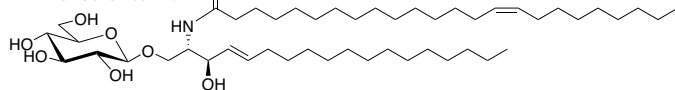
Chemical name: C24:1 Glucosyl(β) Ceramide (d18:1/24:1(15Z))

Formula: $\text{C}_{48}\text{H}_{91}\text{NO}_8$

Molecular weight: 810.24 g/mol

Solubility: 5 mg/ml isopropanol

Chemical structure:



METHODS

Preparation of stock suspension (5 mg/ml)

- Add 1 ml of isopropanol (**not provided**) to 5 mg of β -GlcCer.
- Heat at 60°C for 1 minute, sonicate for 20 seconds, and then vortex until completely dissolved.
- Use immediately or store at -20°C .
- Following storage at -20°C , allow to reach room temperature and vortex before use.
- Prepare dilutions with isopropanol.

Working concentration: 1-10 $\mu\text{g/ml}$

Mincle activation by β -GlcCer using cellular assays

β -GlcCer-induced activation can be studied using HEK-Blue™ Mincle reporter cells, which are HEK293-derived cells stably transfected with the Mincle gene and other genes of the Mincle-NF- κ B signaling pathway. They also stably express an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Mincle activation is assessable by measuring SEAP activity, using QUANTI-Blue™ Solution, a SEAP detection reagent.

For more information visit: <https://www.invivogen.com/hek-blue-clr>.

Day 1

1. Dispense 20 μl of the β -GlcCer suspension at various concentrations (1-10 $\mu\text{g/ml}$ final concentration) per well of a 96-well plate.
2. Ensure that the β -GlcCer suspension is evenly distributed on the surface of the well.
3. Allow to dry for 1 hour at room temperature ($15-25^{\circ}\text{C}$).
4. Prepare a cell suspension (~280,000 cells per ml) and add 180 μl of this suspension (~50,000 cells) to each β -GlcCer-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 data sheet.
2. Add 180 μl of QUANTI-Blue™ Solution to each well containing 20 μl of supernatant.
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	Description	Cat. Code
HEK-Blue™ hMincle	Mincle reporter cells	Inquire
HEK-Blue™ mMincle	Mincle reporter cells	hkb-mmcl
GlcC ₁₄ C ₁₈	Mincle ligand	tlrl-gcc
TDB (Trehalose-6,6-dibehenate)	Mincle ligand	tlrl-tdb
TDM (Trehalose-6,6-dimycolate)	Mincle ligand	tlrl-tdm
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

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 Hong Kong: +852 3622-3480

E-mail: info@invivogen.com