

β -GlcCer

C24:1 Glucosyl(β) Ceramide; Mincle ligand

Catalog code: tlrl-bglcer

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

For research use only

Version 23F22-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 (Fc 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

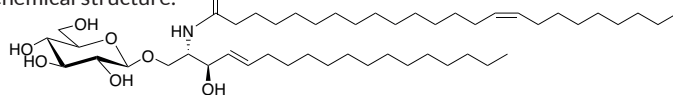
Chemical name: C24:1 Glucosyl(β) Ceramide (d18:1/24:1(15Z))

Formula: C₄₈H₉₁NO₈

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 μ 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 μ l of the β -GlcCer suspension at various concentrations (1-10 μ 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°C).
4. Prepare a cell suspension (~280,000 cells per ml) and add 180 μ l 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% CO₂.

Day 2

1. Prepare QUANTI-Blue™ Solution following the instructions on the data sheet.
2. Add 180 μ l of QUANTI-Blue™ Solution to each well containing 20 μ l 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
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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