

Furfurman

Malassezia furfur cell wall preparation; Dectin-2 agonist

Catalog # ttrl-ffm

For research use only

Version # 14K20-MT

PRODUCT INFORMATION

Contents:

- 10 mg furfurman
- 1.5 ml endotoxin-free water

Storage and stability:

- Product is provided as a solid and shipped at room temperature. Store at 2-8 °C. Solid product is stable for 1 year when properly stored.
- Upon resuspension, store at 2-8 °C. Resuspended product is stable for 1 month when properly stored.

Quality control:

- The biological activity is tested using HEK-Blue™ Dectin-2 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Furfurman is a cell wall preparation of *Malassezia furfur* extracted using a mixture of chloroform and methanol. *Malassezia furfur* is an opportunistic skin fungal pathogen that is recognized by the C-type lectin receptors, in particular Dectin-2¹. This receptor plays an important role in antifungal innate immunity. Dectin-2 binds to mannose carbohydrates, such as the mannose-capped lipoarabinomannan of mycobacteria² and α -mannans found in *Candida albicans*³. Upon binding to its ligand, Dectin-2 couples with the Fc receptor gamma chain⁴ and signals through the kinase Syk and the adaptors CARD9/Bcl-10/MALT1, triggering the activation of NF- κ B and the subsequent production of pro-inflammatory cytokines⁵. Furfurman selectively activates Dectin-2, although at high concentrations it can also activate Dectin-1. Indeed, activation of Dectin-2 by furfurman depends on the presence of terminal α -mannose caps in the latter, as demonstrated by treatment of furfurman with an exo- α -mannosidase, an enzyme that removes mannose groups.

1. Ishikawa T. *et al.*, 2013. Identification of distinct ligands for the C-type lectin receptors Mincle and Dectin-2 in the pathogenic fungus *Malassezia*. *Cell Host Microbe*. 13(4):477-88. 2. Yonekawa A. *et al.*, 2014. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. *Immunity*. 41(3):402-13. 3. Saijo S. *et al.*, 2010. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. *Immunity*. 32(5):681-91. 4. Sato K. *et al.*, 2006. Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. *J Biol Chem*. 281(50):38854-66. 5. Sancho D. & Reis e Sousa C., 2012. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annu Rev Immunol*. 30:491-529.

METHOD

Preparation of stock solution (10 mg/ml)

1. Add 1 ml endotoxin-free water to 10 mg furfurman.
2. Vortex for 30 seconds.

Note: The suspension appears turbid.

3. Store at 2-8 °C.

Note: Further dilutions can be prepared by adding endotoxin-free water.

Dectin-2 activation using furfurman

Furfurman was tested in HEK-Blue™ hDectin-2, a cell line derived from HEK293 that was transfected with the human Dectin-2 gene and other genes from the Dectin-2 signaling pathway. These cells also stably express an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene.

Day 1

1. Dispense 20 μ l of a furfurman suspension at various concentrations (0.1 to 10 μ g/ml) per well in a 96-well plate.
2. Ensure that the furfurman suspension is evenly distributed on the surface of the well.
3. Allow to dry for 1 hour at 70 °C.
4. Prepare a cell suspension (~280,000 cells per ml) and add 180 μ l of this suspension (~50,000 cells) to each furfurman-containing well.
5. Incubate the cells for 20-24 hours at 37 °C and 5% CO₂.

Day 2

6. Prepare QUANTI-Blue™ following the instructions on the pouch.
7. Add 150 μ l of QUANTI-Blue™ to each well in a 96-well plate.
8. Add 50 μ l of supernatant to each well containing QUANTI-Blue™.
9. Incubate the plate at 37 °C for 30 minutes to 6 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
Anti-hDectin-2-IgG	mabg-hdect2
HEK-Blue™ hDectin-2 cells	hkb-hdect2
pUNO-hDectin2	puno-hdectin2
pNiFty2-SEAP	pnifty2-seap
QUANTI-Blue™	rep-qbl

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