

PRODUCT DATA SHEET

Trehalose 6,6'-dimycolate [Cord Factor] Endotoxin-free (sterile)

Cat. No.: IAX-200-101

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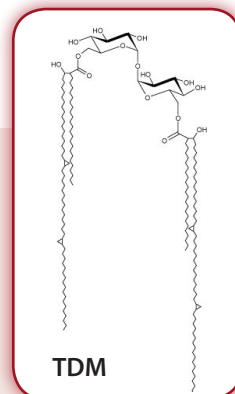
NAME: Cord Factor, TDM.
SOURCE: Isolated from *Mycobacterium tuberculosis*.
CAS: 61512-20-7.
FORMULATION: Lyophilised. Sterile.
PURITY: Single spot (TLC).
ENDOTOXIN-FREE: 0.0002EU/μg.

SOLUBILITY: Soluble (5mg/ml) in chloroform:methanol:water (vol:vol) 90:9:1, hexane or isopropanol.

HANDLING/ACTIVITY: To stimulate mouse bone marrow-derived macrophages, Cord Factor was suspended at a concentration of 0.2mg/ml in hexane. Of the resulting solution 1 or 10μg/well were layered in 96-well tissue culture plates and the solvent completely evaporated. Control wells were layered with solvent without Cord Factor and also incubated at 37°C. To this layer of Cord Factor, bone marrow-derived macrophages were added in 100μl of medium and incubated at 37°C for 24 hours before activation e.g. TNF-α production was measured in the supernatant.
In vivo pulmonary granuloma formation in mice can be induced by 10μg Cord Factor per mouse applied i.v. in a water/oil/water emulsion.

SHIPPING: Ambient.
STORAGE: 4°C. After reconstitution in water prepare aliquots and store at -20°C (shelf-life 6 months). Avoid freeze/thaw cycles. After thawing stable for one day at 4°C, do not freeze again.

STABILITY: 2 years after receipt as supplied.



General Information:

The mycobacterial glycolipid trehalose-6,6'-dimycolate (TDM), also named Cord Factor (CF), is an important regulator of immune responses during *Mycobacterium tuberculosis* (Mtb) infections. Macrophages recognize TDM through the Mincle receptor and initiate TDM-induced inflammatory responses, leading to lung granuloma formation. Controlled use of its cell wall activates macrophages in ways that can be harnessed for therapy. For example, *M. bovis* Bacille Calmette-Guérin (BCG) is one of the most widely used antitumor adjuvant therapies in humans. Freund's adjuvant, an emulsion of mycobacterial cell wall components in paraffin oil, is mixed with antigens to optimize memory T and B cell responses in mice. TDM along with a detoxified derivative of Lipid A (MPLA) and cell wall skeleton make up a formulation also known under the name of Ribi adjuvant. Recent studies suggest that Mincle is a pivotal receptor for the mycobacterial cord factor. However, additional receptors may bind TDM independently or in cooperation with Mincle. Candidates include other CLEC proteins, such as Dectin-2, which also associates with FcRγ, is expressed in macrophages, and binds to Mtb. The scavenger receptor MARCO interacts with TDM, yet lacks an intracellular domain for signal initiation. In contrast, Mincle can directly trigger Syk-Card9 signalling via its association with FcRγ. Whereas in the absence of Mincle macrophages did not respond to TDM, a recent report found Mincle-deficient mice capable of mounting an efficient granulomatous and protective immune response after low and high dose infections with Mtb. Mutant mice generated a normal T helper (TH)₁ and TH₁₇ immune response followed by the induction of efficient macrophage effector mechanisms and control of mycobacterial growth identical to wildtype mice. The absence of the innate receptor Mincle may be fully compensated for *in vivo* in terms of sensing Mtb and mounting a protective inflammatory immune response.

References:

- [1] The chemical structure of the cord factor of *Mycobacterium tuberculosis*. Noll H, et al. *Biochim. Biophys. Acta* (1956); 20:299
- [2] Studies of a biochemical lesion in experimental tuberculosis in mice. 8. Effect of derivatives and chemical analogues of cord factor on structure and function of mouse liver mitochondria. Kato M, et al. *Am. Rev. Respir. Dis.* (1968); 98:668
- [3] Granuloma formation induced in mice by chemically defined mycobacterial fractions. Bekierkunst A, et al. *J. Bacteriol.* (1969); 100:95
- [4] MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and *Mycobacterium tuberculosis*. Bowdish DM, et al. *PLoS Pathog.* (2009); 5:e1000474
- [5] Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. Ishikawa E, et al. *J. Exp. Med.* (2009); 206:2879
- [6] Mincle is essential for recognition and adjuvant activity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. Schoenen H, et al. *J. Immunol.* (2010); 184:2756
- [7] Mincle is not essential for controlling *Mycobacterium tuberculosis* infection. Heitmann L, et al. *Immunobiology* (2012); ahead of print

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