

TDB VacciGrade™

Trehalose-6,6-dibehenate; Mincle based-adjuvant

Catalog code: vac-tdb

<https://www.invivogen.com/tdb-vaccigrade>

For research use only. Not for use in humans.

Version 19H30-MM

PRODUCT INFORMATION

Contents

- 2 x 1 mg TDB VacciGrade™
- 10 ml sterile endotoxin-free physiological water (NaCl 0.9%)

Storage and stability

- TDB VacciGrade™ is provided as a sterile powder and shipped at room temperature. Store at -20 °C. Lyophilized product is stable for 1 year at -20 °C.

Quality control

TDB VacciGrade™ is a preclinical grade. It is prepared under strict aseptic conditions and is tested for the presence of endotoxins. TDB VacciGrade™ is guaranteed sterile and its endotoxin level is <5 EU/mg.

DESCRIPTION

Trehalose-6,6-dibehenate (TDB) is a non-toxic synthetic analogue of the mycobacterial cell wall component trehalose 6,6' dimycolate (TDM, also known as cord factor). TDB was recently shown to rely on the C-type lectin Mincle and the signaling molecules Syk and Card9 to trigger a Th1/Th17 innate immunity¹. Incorporation of TDB combined with the synthetic amphiphilic cationic lipid compound dimethyldioctadecylammonium (DDA) into liposomes, known as CAF01, has been shown to strongly enhance cellular and humoral responses against a protein antigen². Adjuvanticity of the cationic DDA:TDB liposomes and sustained protection against disease challenge has been demonstrated in particular with a tuberculosis vaccine candidate^{3,4}, and has good potential for application in a range of other diseases⁵.

1. 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. **2. Davidsen, J. et al., 2005.** Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from *M. tuberculosis* (trehalose 6,6'-dibehenate)-a novel adjuvant inducing both strong CMI and antibody responses. *Biochim. Biophys. Acta.* 1718:22–31. **3. Holten-Andersen L. et al., 2004.** Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. *Infect. Immun.* 72:1608–1617. **4. Christensen D. et al., 2011.** Cationic liposomes as vaccine adjuvants. *Expert Rev Vaccines.* 10(4):513-21. **5. Agger EM. et al., 2008.** Cationic liposomes formulated with synthetic mycobacterial cordfactor (CAF01): a versatile adjuvant for vaccines with different immunological requirements. *PLoS One.* 3(9):e31116.

CHEMICAL PROPERTIES

CAS number: 66758-35-8

Formula: C₅₆H₁₀₆O₁₃

Molecular weight: 987.43 g/mol

Working Concentration: 1 - 100 µg/mouse

METHODS

Preparation of stock suspension (1 mg/ml)

- Add 100 µl DMSO to 1 mg TDB VacciGrade™, heat at 60 °C (approx. 15-30 seconds) and vortex.
- Once resuspended, immediately add 900 µl sterile physiological water (provided) or phosphate buffered saline (PBS without Ca²⁺ and Mg²⁺), 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. Resuspended product can be stored at 4 °C for 6 months. Prior to each use, bring suspension to room temperature and homogenize by vortexing for 30 seconds.

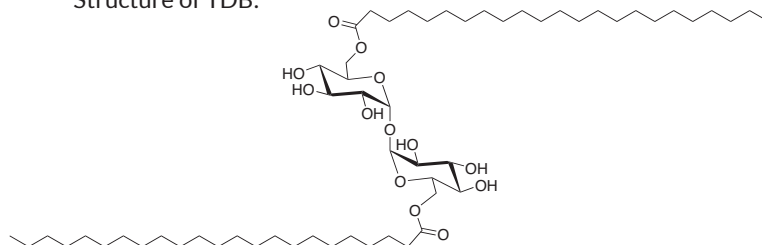
OR

Preparation of DDA:TDB complex

The protocol described below is based on the method described in Henriksen-Lacey M. *et al.*, 2010. Liposomes based on dimethyldioctadecylammonium promote a depot effect and enhance immunogenicity of soluble antigen. *J Control Release.* 142(2):180-6.

- Prepare a 5 mg/ml solution of dimethyldioctadecylammonium bromide (DDA; not provided) in chloroform/methanol (ratio 9:1 v/v).
- Prepare a 5 mg/ml solution of TDB VacciGrade™ (dissolve 1 mg TDB VacciGrade™ using 0.2 ml chloroform/methanol (ratio 9:1 v/v)).
- Add 1 ml DDA solution (5 mg/ml) to 0.2 ml TDB VacciGrade™ solution (5 mg/ml) providing a ratio of 5:1 w/w (DDA:TDB).
- Use a roto-evaporator to remove the chloroform/methanol followed by flushing with N₂, to produce a thin lipidic film containing the DDA:TDB complex.
- Store DDA:TDB complex at -20 °C for 3 months or use immediately.
- Hydrate the lipidic film with 2 ml TRIS base buffer (10 mM; pH 7.4). Heat the suspension for 20 minutes at 60 °C. Allow to cool before use. This suspension should not be stored, use within 1 day.

Structure of TDB:



TECHNICAL SUPPORT

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RELATED PRODUCTS

Product	Description	Catalog Code
Alum and Emulsions		
AddaVax™	Squalene-Oil-in-water	vac-adx-10
Alhydrogel 2%	Aluminium hydroxide gel	vac-alu-250
CFA	Complete Freund's adjuvant	vac-cfa-10
IFA	Incomplete Freund's adjuvant	vac-ifa-10
PRR Ligands		
c-di-AMP VacciGrade™	STING agonist	vac-nacda
c-di-GMP VacciGrade™	STING agonist	vac-nacdg
2'3'-cGAMP VacciGrade™	STING agonist	vac-nacga23
Flagellin FliC VacciGrade™	TLR5 agonist	vac-fla
Imiquimod VacciGrade™	TLR7 agonist	vac-imq
MPLA-SM VacciGrade™	TLR4 agonist	vac-mpla
MPLAs VacciGrade™ (synthetic MPLA)	TLR4 agonist	vac-mpls
N-glycolyl-MDP VacciGrade™	NOD2 agonist	vac-gmdp
ODN 1585 VacciGrade™	Murine TLR9 agonist	vac-1585-1
ODN 1826 VacciGrade™	Murine TLR9 agonist	vac-1826-1
ODN 2006 VacciGrade™	Human TLR9 agonist	vac-2006-1
Pam3CSK4 VacciGrade™	TLR2 agonist	vac-pms
Poly(I:C) (HMW) VacciGrade™	TLR3 agonist	vac-pic
OVA Antigens		
EndoFit™ Ovalbumin	For <i>in vivo</i> use; endotoxin level <1EU/mg	vac-pova
Ovalbumin	For detection; Western, ELISA	vac-stova
Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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