Certificate of Analysis for NR-51683

Enterotoxigenic *Escherichia coli* Double Mutant Heat-Labile Toxoid (dmLT), Adjuvant-Active, Recombinant from *Escherichia coli*

Catalog No. NR-51683

This reagent is the tangible property of the U.S. Government.

Product Description:

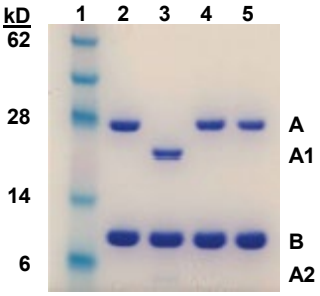
NR-51683 is a recombinant toxoid of enterotoxigenic *Escherichia coli (E. coli)* (ETEC) heat-labile toxin (LT) with a double genetic mutation (R192G/L211A; based on the recombinant sequence) which renders the protein non-toxic yet adjuvant-active. The recombinant double mutant, dmLT or LT(R192G/L211A), was expressed in *E. coli* and purified by immobilized galactose chromatography.

Lot: 70026501[1] **Manufacturing Date: APR2019**

TEST	SPECIFICATIONS	RESULTS
SDS-PAGE (Figure 1)[2]	Protein bands of interest represents > 95% of total staining intensity above background Trypsin insensitive	Protein bands of interest represents > 95% of total staining intensity above background Trypsin insensitive
Functional Activity Western blot Oral adjuvanticity with Tetanus toxoid by ELISA	 Reactive Confirmed	 Reactive Confirmed (Figure 2)[3]
Induction of cAMP in T84 Cells (3 h) (Figure 3)[4] Native LT dmLT (NR-51683)	 Report results Report results	 ~ 130 pmol/mL cAMP at 0.001 µg LT ~ 50 pmol/mL cAMP at 1 µg dmLT
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered
Endotoxin Content[5]	Report results	< 1 EU per mg

[1]This item was manufactured and subjected to quality control testing by Tulane University School of Medicine, New Orleans, Louisiana, USA.
[2]Trypsin-mediated cleavage of the A-subunit into A1 (21 kDa) and A2 (7 kDa) is required for activation of LT and is a key factor that distinguishes LT from single mutant mLT(R192G). dmLT exhibits no trypsin-mediated cleavage of the A-subunit into A1 but is more sensitive than either LT or mLT(R192G) to complete and rapid degradation; see, Norton, E. B., et al. "Characterization of a Mutant *Escherichia coli* Heat-Labile Toxin, LT(R192G/L211A), as a Safe and Effective Oral Adjuvant." Clin. Vaccine Immunol. 18 (2011): 546-551. PubMed: 21288994.
[3]dmLT boosts sera anti-Tetanus toxoid (TT) IgG responses, indicating maintenance of oral adjuvanticity.
[4]In human colorectal carcinoma (T84) cells, 1 µg of dmLT induces less cAMP than 0.001 µg of native LT, indicating detoxification of enterotoxicity.
[5]Limulus Amoebocyte Lysate Assay (LAL)

Figure 1: SDS-PAGE Analysis



Lane 1: MW markers (kD)
Lane 2: Untrypsinized LT
Lane 3: Trypsinized LT
Lane 4: Untrypsinized dmLT
Lane 5: Trypsinized dmLT

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Figure 2: Oral Adjuvanticity with Tetanus Toxoid by ELISA

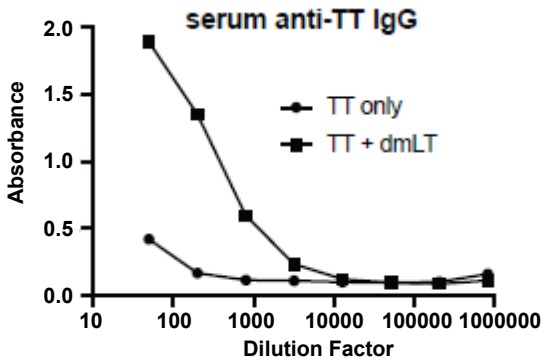
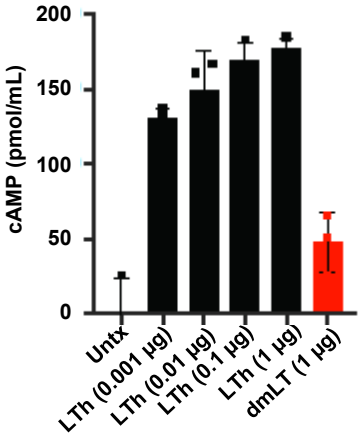


Figure 3: Induction of cAMP in T84 Cells



/Heather Couch/
Heather Couch 14 AUG 2019
Program Manager or designee, ATCC Federal Solutions

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