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.

**For research use only. Not for human use.**

**Contributor and Manufacturer:**
Jacob P. Bitoun, Ph.D., Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana, USA

**Product Description:**
NR-51683 is a recombinant toxoid of enterotoxigenic *Escherichia coli* (*E. coli*) (ETEC) heat-labile toxin (LT) with a double mutation (R192G/L211A; based on the recombinant sequence). The recombinant double mutant, dmLT or LT(R192G/L211A), has reduced proteolytic activation and enterotoxicity while maintaining adjuvanticity equal to native LT. **NR-51683** was expressed in *E. coli* and purified by immobilized galactose chromatography. The theoretical molecular weight is approximately 86 kilodaltons.

The ETEC infectious process is initiated by the organism adhering to the host intestinal epithelial cells via interactions between bacterial adhesions, colonization factors (including colonization factor antigens (CFAs), coli surface (CS), and putative colonization factors (PCFs)) and host receptors. Similar to the closely related cholera toxin, LT is a multimeric molecule comprised of an A subunit and five B subunits. The pentameric B subunit is essential for binding the toxin to ganglioside GM1 in host cell membranes, and a single A subunit, which needs to be nicked by proteolysis and reduced, yields an A1-"enzyme" and an A2-"linker" peptide. A1 is then translocated across the cell membrane, possibly after endocytosis, upon which it ADP-ribosylates the G protein Go. The amino acid sequence of both subunit A and subunit B of heat-labile toxin have been determined (GenPept: P06717 and P32890, respectively). The crystal structure of heat-labile toxin complexed with lactose has been determined to 2.3 Å (PDB: 1LTT).

**Material Provided:**
Each vial of NR-51683 contains approximately 3 mg of purified recombinant dmLT adjuvant, which was lyophilized from TEAN (Tris, EDTA, sodium azide and sodium chloride) buffer. Resuspend slowly with 1.0 mL of HyClone water and gently mix by hand to dissolve. The solution may appear slightly turbid.

**Packaging/Storage:**
NR-51683 was packaged aseptically in glass vials, lyophilized and sealed under vacuum. The product is provided at room temperature and should be stored at 2°C to 8°C immediately upon arrival. Freeze-thaw cycles should be avoided.

**Functional Activity:**
NR-51683 is functional in SDS-PAGE, cAMP assays in human colorectal carcinoma (T84) cells, western blot and oral adjuvanticity (ELISA) assays. The novel dmLT adjuvant promotes dose sparing, mucosal immunity and longevity of antibody responses to the inactivated polio vaccine in a murine model.

**Citation:**
Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: Enterotoxigenic *Escherichia coli* Double Mutant Heat-Labile Toxoid (dmLT), Adjuvant-Active, Recombinant from *Escherichia coli*, NR-51683.”

**Biosafety Level: 1**

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NR-51683 is claimed in U.S. Patent Numbers 6,019,982 and 6,033,673, and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.¹

References:

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