

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

**Catalog No. NR-51682**

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-51682 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.<sup>1,2</sup> NR-51682 was expressed in *E. coli* and purified by immobilized galactose chromatography.<sup>1</sup> 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.<sup>3</sup> ETEC then causes secretory diarrhea by expressing LT and/or heat-stable enterotoxin (STh).<sup>4</sup> 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 G<sub>M1</sub> 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 G<sub>sα</sub>.<sup>5</sup>

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: [1LTI](#)).<sup>5</sup>

**Material Provided:**

Each vial of NR-51682 contains approximately 2 mg of purified recombinant dmLT adjuvant, which was lyophilized from TEAN (Tris, EDTA, sodium azide and sodium chloride) buffer.<sup>1</sup>

**Note:** 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-51682 was packaged aseptically in glass vials, lyophilized and sealed under vacuum.<sup>1</sup> 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-51682 is functional in SDS-PAGE, cAMP assays in human colorectal carcinoma (T84) cells, western blot and oral adjuvanticity (ELISA) assays.<sup>1,2</sup> The novel dmLT adjuvant promotes dose sparing, mucosal immunity and longevity of antibody responses to the inactivated polio vaccine in a murine model.<sup>1,2</sup>

**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-51682."

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. [Biosafety in Microbiological and Biomedical Laboratories](#). 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

**Disclaimers:**

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at [www.beiresources.org](http://www.beiresources.org).

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

**Use Restrictions:**

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

NR-51682 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.<sup>1</sup>

**References:**

1. Bitoun, J. P., Personal Communication.
2. 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. Beachey, E. H. "Bacterial Adherence: Adhesin-Receptor Interactions Mediating the Attachment of Bacteria to Mucosal Surface." J. Infect. Dis. 143 (1981): 325-345. PubMed: 7014727.
4. Yamamoto, T. and T. Yokota. "Plasmids of Enterotoxigenic *Escherichia coli* H10407: Evidence for Two Heat-Stable Enterotoxin Genes and a Conjugal Transfer System." J. Bacteriol. 153 (1983): 1352-1360. PubMed: 6298182.
5. Sixma, T. K., et al. "Lactose Binding to Heat-Labile Enterotoxin Revealed by X-Ray Crystallography." Nature 355 (1992): 561-564. PubMed: 1741035.
6. Toprani, V. M., et al. "Structural Characterization and Physicochemical Stability Profile of a Double Mutant Heat Labile Toxin Protein Based Adjuvant." J. Pharm. Sci. 106 (2017): 3474-3485. PubMed: 28780391.

ATCC® is a trademark of the American Type Culture Collection.

