

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

**Catalog No. NR-51681**

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**Contributor and Manufacturer:**

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**Product Description:**

NR-51681 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-51681 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 GM<sub>1</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>sa</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: [1LTT](#)).<sup>3</sup>

**Material Provided:**

Each vial of NR-51681 contains approximately 1 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-51681 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-51681 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-51681.”

**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/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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NR-51681 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.
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