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

**Certificate of Analysis for NR-51681**

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

**Product Description:**

NR-51681 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:** 70026499

**Manufacturing Date:** APR2019

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

1This item was manufactured and subjected to quality control testing by Tulane University School of Medicine, New Orleans, Louisiana, USA.

2Trypsin-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.

3dmLT boosts sera anti-Tetanus toxoid (TT) IgG responses, indicating maintenance of oral adjuvanticity.

4In human colorectal carcinoma (T84) cells, 1 µg of dmLT induces less cAMP than 0.001 µg of native LT, indicating detoxification of enterotoxicity.

5Limulus Amoebocyte Lysate Assay (LAL)

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**Figure 1: SDS-PAGE Analysis**

![SDS-PAGE Analysis](image)
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Figure 2: Oral Adjuvanticity with Tetanus Toxoid by ELISA

- Serum anti-TT IgG
- TT only
- TT + dmLT

Figure 3: Induction of cAMP in T84 Cells

/Heather Couch/
Heather Couch 13 AUG 2019
Program Manager or designee, ATCC Federal Solutions

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