b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-50761

## Heat-Stable Enterotoxin (STh) from Enterotoxigenic *Escherichia coli*

## Catalog No. NR-50761

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

**Product Description:** NR-50761 is a preparation of the human variant of heat-stable enterotoxin (STh) purified from enterotoxigenic *Escherichia coli* (*E. coli*) (ETEC). The protein was purified from the culture supernatant by tangential flow filtration, hydrophobic interaction chromatography (HIC), gel filtration chromatography, and high-pressure reverse phase liquid chromatography.

**Lot[1]: 002042017** **Manufacturing Date: 01MAY2017**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Appearance** | Report results | Clear |
| **Purity**<br>SDS-PAGE<br><br><br>RP-HPLC (C18 column) | <br>2,048 Da band represents > 95% of total staining intensity above background<br>Report results | <br>2,048 Da band represents > 99% of total staining intensity above background<br>STh represented by single peak in elution profile |
| **T84 cGMP Assay[2]**<br>NR-50761 (25 ng) | <br>Report results | <br>1368 pmol/mL cGMP produced |
| **Concentration[3]** | Report results | 2.0 mg/mL ± 0.04 mg/mL in 0.5 mL |
| **Endotoxin Content (Limulus Amoebocyte Lysate Assay)[4]** | Report results | > 1 EU/mL |
| **Sterility** | 0.22 µm filter sterilized | 0.22 µm filter sterilized |

[1]Production and quality control were completed by Jacob P. Bitoun, Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana, USA. All test data was obtained post-freeze.

[2]Human colonic T84 cells were grown to 80-100% confluency in 24-well tissue culture plates and washed once with serum free DMEM/F-12, pre-treated with phosphodiesterase inhibitors zardaverine (20 µM) and vardenafil (30 µM) in DMEM/F-12 plus 1% FBS for one hour at 37°C in 5% $CO_2$ prior to toxin administration. STh was diluted to 12.5 ng/µL and two microliters (25 ng STh) was applied to a single well of T84 cells in triplicate. T84 cells were incubated with toxin for 2 hours prior to extensive washing with ice cold PBS. Cells were lysed and cGMP was measured using the cGMP Parameter Kit (#KGE003, R&D Systems, Minneapolis, MN). Assays were completed in triplicate.

[3]Concentration was determined using the Warburg-Christian method [Warburg, O. and Christian, W. Biochem. Z. 310: 384 (1941)]

[4]QCL-1000™ Assay (Cat. No. 50-647U) from Lonza.

**Date:** 30 JUN 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

Support Provided by NIAID

**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898