b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50764

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

Catalog No. NR-50764

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

Product Description: NR-50764 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]: 005042017 **Manufacturing Date: 01MAY2017**

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	Clear
Purity SDS-PAGE RP-HPLC (C18 column)	 2,048 Da band represents > 95% of total staining intensity above background Report results	 2,048 Da band represents > 99% of total staining intensity above background STh represented by single peak in elution profile
T84 cGMP Assay[2] NR-50764 (25 ng)	 Report results	 1309 pmol/mL cGMP produced
Concentration[3]	Report results	0.5 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

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