

**Staphylococcal Enterotoxin B Toxoid, Recombinant from *Escherichia coli***

**Catalog No. NR-10049**

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

**Product Description:** NR-10049 is a recombinant toxoid of Staphylococcal Enterotoxin B (SEB) with genetic mutations (L56R, Y100A, Y105A; based on the recombinant sequence) which render the protein non-toxic. NR-10049 contains a hexa-histidine tag near the N-terminus. The recombinant protein was expressed in *Escherichia coli* and purified by nickel affinity chromatography.

**Lot: 63487424**

**Manufacturing Date: 05MAY2015**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
<b>SDS-PAGE (SYPRO Orange Densitometer Scan)</b>	Protein band of interest represents > 95% of total staining intensity above background	Protein band of interest represents > 95% of total staining intensity above background (Figure 1)
<b>SELDI-TOF Mass Spectrometry</b>	Measured mass within 5% of expected mass: 29945 daltons	Measured mass (30024 daltons) within 0.3% of expected mass
<b>SELDI-TOF Mass Spectrometry of Lys-C Proteolysis Products</b>	> 50% of total residues accounted for in peptides of expected mass	53% of total residues accounted for in peptides of expected mass
<b>Concentration by Bicinchoninic Acid Protein Assay<sup>1</sup></b>	1.0 mg per mL ± 5%	1.0 mg per mL
<b>Functional Activity by Western blot<sup>2</sup> (Figure 2)</b> Staphylococcal enterotoxin B toxoid Carbonic anhydrase	Reactive Not reactive	Reactive Not reactive
<b>T Lymphocyte Activation</b> Human interferon-γ ELISPOT assay <sup>3,4</sup> NR-10049 (33.3 nM) Staphylococcal enterotoxin B (33.3 nM) Negative control (cells with no antigen)	Report results Positive T-cell response Negative T-cell response	< 10 spots per sample > 300 spots per sample ≤ 5 spots per sample
<b>Sterility</b>	0.22 μm filter sterilized	0.22 μm filter sterilized
<b>Absorbance Ratio (OD<sub>280</sub>/OD<sub>260</sub>)</b>	Report results	1.6
<b>Endotoxin Content<sup>5</sup></b>	Report results	< 125 EU per mg

<sup>1</sup>Using Pierce™ BCA Protein Assay Kit with BSA standard curve

<sup>2</sup>Using anti-Staphylococcal Enterotoxin B polyclonal antibody produced in rabbit (SIGMA® S9008)

<sup>3</sup>Measured by Mabtech human IFN-gamma ELISpot

<sup>4</sup>The assay analyzes interferon-γ production at a single cell level. Activation of large numbers of naïve T lymphocytes is characteristic of bacterial superantigens such as SEB, and the abrogation of this response confirms the inactivation of NR-10049.

<sup>5</sup>Measured by Limulus Amoebocyte Lysate Assay (LAL)

# Certificate of Analysis for NR-10049

Figure 1 - SDS-PAGE

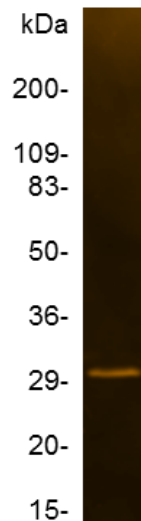
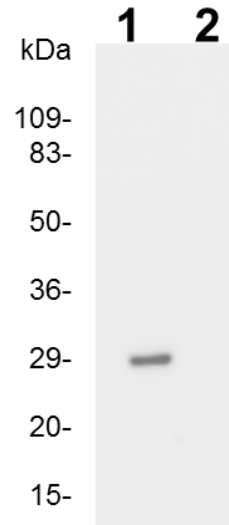


Figure 2 - Western Blot



Lane 1: NR-10049 (1  $\mu$ g)  
Lane 2: Carbonic Anhydrase (1  $\mu$ g)

Date: 29 JUN 2015

Signature:

BEI Resources Authentication

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

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