

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

**Catalog No. NR-10049**

This reagent is the 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: 58152420**

**Manufacturing Date: 10MAY2008**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Clear with no particulate matter	Clear with no particulate matter
<b>SDS-PAGE (SYPRO Orange densitometer scan)</b>	Dominant band ~ 30 kDa > 95% pure	Dominant band of ~ 30 kDa > 95% pure (Figure 1)
<b>SELDI-TOF Mass Spectrometry</b>	Measured mass within 5% of expected mass based on amino acid sequence: 29,945 daltons	Measured mass within 0.1% of expected mass based on amino acid sequence: 29,964 daltons
<b>SELDI-TOF Mass Spectrometry of Lys-C Proteolysis Products</b>	> 50% of total residues accounted for in peptides of expected mass	61% of total residues accounted for in peptides of expected mass
<b>Concentration by Bicinchoninic Acid Protein Assay</b>	Report results	1.0 mg/mL
<b>Functional Activity</b> Western blot <sup>1</sup> (Figure 2) Staphylococcal enterotoxin B toxoid Carbonic anhydrase	Reactive Not reactive	Reactive Not reactive
<b>T Lymphocyte Activation</b> Human interferon- $\gamma$ ELISPOT assay <sup>2,3</sup> NR-10049 (300 nM) Staphylococcal enterotoxin B (3 nM) Negative control (cells with no antigen)	Report results Positive T-cell response Negative T-cell response	~ 25 spots per sample > 200 spots per sample ~ 12 spots per sample
<b>Sterility</b>	0.22 $\mu$ m filter sterilized	0.22 $\mu$ m filter sterilized
<b>Absorbance Ratio (OD<sub>280</sub>/OD<sub>260</sub>)</b>	Report results	2.15
<b>Endotoxin Content</b>	Report results	12.5 EU/mg

<sup>1</sup>Using anti-Staphylococcal Enterotoxin B antibody produced in rabbit (SIGMA<sup>®</sup> S9008).

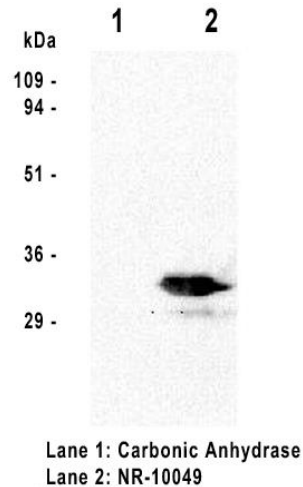
<sup>2</sup>Using Human Interferon- $\gamma$  ELISPOT Kit (Pierce ELHIFNG).

<sup>3</sup>This assay analyzes interferon- $\gamma$  production at a single cell level. A spot forms at the site where secreted cytokine is bound; one spot is equal to one molecule-secreting cell. 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.

Figure 1



Figure 2



Date: 17 SEP 2014

Signature:

Title:

Technical Manager, BEI Authentication or designee

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