

## *S. aureus* recombinant Gamma Hemolysin A (Hlg A)

**Catalog #:** 1403-003

**Lot #:** 1504002

**Description:** Purified, *Staphylococcus aureus* recombinant Gamma Hemolysin A (Hlg A). The rHlg A (tag free) is expressed in *E. coli* and purified by FPLC. The theoretical molecular weight of the protein is 34,956 Daltons.

**Storage:** 2-3 weeks at -20°C, -80°C long term

**Size:** 100 µg of protein is supplied in PBS at a concentration of 2.065 mg/mL. Protein demonstrates a molecular weight of approximately 35 kDa.

**Endotoxin:** 3.332 Endotoxin Units/mg

**Relevance:** This protein may be used in functional toxicity assays in combination with rHlg B, or as a control protein in ELISA assays or Western blotting when detecting toxins produced by different strains of *S. aureus*.

### **Recommended Dilutions:**

**ELISA:** Assay-dependent dilution.

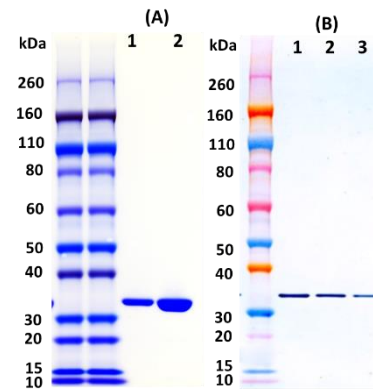
**WB:** Assay-dependent dilution; internal QC demonstrates detection of 50 ng of rHlg A protein using anti-PVL LukS polyclonal antibody (cat# 04-0009) in Western blotting.

**Cytotoxicity assay:** Cytotoxicity can be detected in human neutrophils when used in combination with rHlg B in a concentration range of 4-200 nM.

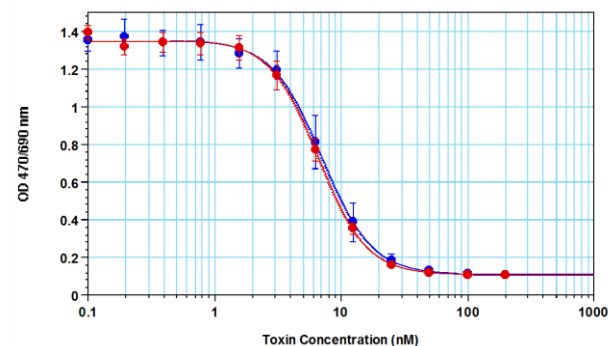
**For additional *S. aureus* products, please visit:**

<http://ibtbioservices.com/index.php/product-and-reagents/staphylococcal-products>

### **SDS-PAGE and Western Blot Detection**



(A) SDS-PAGE of rHlg A: 1 µg (lane 1) and 5 µg (lane 2). (B) Western blot detection of rHlg A at 200 ng, 100 ng and 50 ng (lanes 1-3), using IBT's anti-PVL Luk S polyclonal antibody (Cat# 04-0009) at 0.5 µg/mL and an anti-rabbit IgG-HRP conjugate followed by substrate.



**Toxin Functionality:** Human promyelocytic leukemia cell line HL60 was differentiated into neutrophils by treatment with DMSO. Neutrophils were incubated with serial dilutions of rHlg A and rHlg B at equimolar concentration for 3 hours at 37°C with 5% CO<sub>2</sub> and 95% humidity. Cellular viability was determined by adding XTT and incubation for additional 16 hours. Cells were centrifuged and the OD determined in the supernatants at 470/690nm. EC<sub>50</sub> values were found to be 7.12 nM for the current lot 1504002 (blue circles) and 6.67 nM for the previous lot 1311002 (red circles).

**Intended for research use only. Not for human, therapeutic, or diagnostic applications.**

*The buyer cannot sell or otherwise transfer this product for Commercial Purposes without written approval of Integrated BioTherapeutics, Inc.*

Copyright 2015. Integrated BioTherapeutics, Inc. All rights reserved