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S. aureus recombinant Gamma Hemolysin A (Hlg A)

Catalog #: 1403-003

Lot #: 1805006

Description: Purified, *Staphylococcus aureus* recombinant Gamma Hemolysin A (rHlg 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 plus 5% glycerol, at a concentration of **2.176 mg/mL**. Protein demonstrates a molecular weight of approximately 35 kDa.

Endotoxin: 3.077 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:

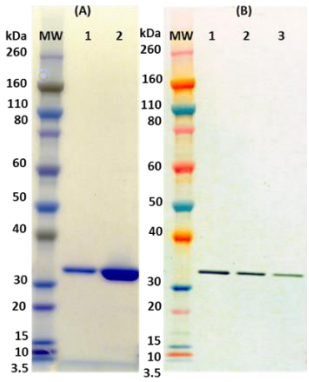
ELISA: Assay-dependent dilution.

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.

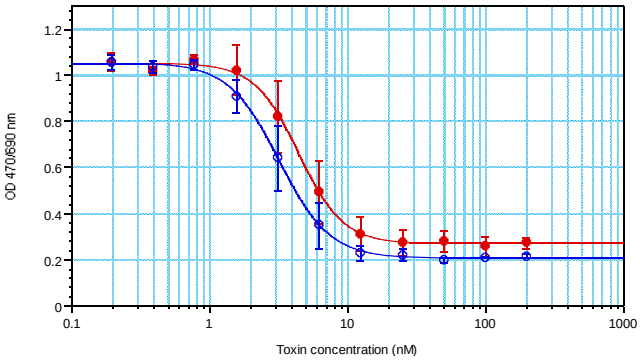
For additional *S. aureus* products, please visit:
http://www.ibtbioservices.com/reagents/staphylococcus/

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 0.05 µg/mL and an anti-rabbit IgG-HRP conjugate followed by TMB membrane 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% CO2 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_{50} values were found to be 4.39 nM for the current lot 1805006 (red circles) and 3.16 nM for the previous lot 1504002 (blue circles).

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