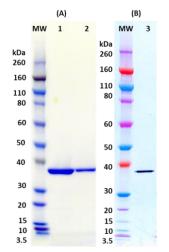
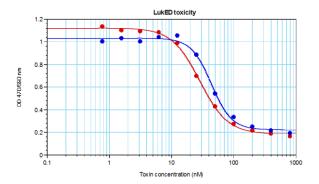


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SDS-PAGE and Western Blot Detection



(A) SDS-PAGE of rLukD: 5 μ g (lane 1) and 1 μ g (lane 2). (B) Western blot detection of rLukD at 100 ng (lane 3), using IBT's anti-LukF-PV polyclonal antibody (cat# 0312-002) at 0.5 μ g/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 rLukE (cat# 0530-004, lot 1503005) and rLukD 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 470/690 nm. Red circles represent the current lot 1707001 and blue circles represent the previous lot 1508003. EC50 were found to be 28.8 nM for the current lot and 44.2 nM for the previous lot.

S. aureus recombinant LukD

Catalog #: 0530-003

Lot #: 1707001

Description: Purified, *Staphylococcus aureus* recombinant leukocidin-D (rLukD). The rLukD (tag free) is expressed in *E. coli* and purified by FPLC. The theoretical molecular weight of the protein is 36,889 Daltons.

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

Size: 100 μ g of protein is supplied in PBS at a concentration of **2.224 mg/mL**. Protein demonstrates a molecular weight of approximately 37 kDa.

Endotoxin: 4.232 Endotoxin units/mg

Relevance: This protein may be used in functional toxicity assays in combination with rLukE, 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 100 ng of rLukD protein using anti-PVL LukF polyclonal antibody (cat# 0312-002) in Western blotting.

Cytotoxicity assay: Cytotoxicity can be detected in human neutrophils when used in combination with LukE.

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

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