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Product #172L
Lot #1722B15A2
Release Date: July 2017

CERTIFICATE OF ANALYSIS
ANTHRAX LETHAL FACTOR (LF) RECOMBINANT
from *Bacillus anthracis*
Lot #1722B15A2

Contents

Each vial contains 1 mg of lethal factor from *Bacillus anthracis* in 0.5 mL of 5 mM HEPES, 50 mM NaCl, pH 7.5. **Handle the product gently. Do not vortex.**

Packaging/Storage

This product is supplied as an aseptically packaged liquid. Store at -20°C.

Concentration

Protein concentration was determined by absorbance at 280 nm using an extinction coefficient of 0.88 for a 1 mg/mL solution. This value is calculated by ProtParam[1] using an algorithm based on the Edelhoch[2] method with modifications described in Pace et al.[3]

Protein concentration was also confirmed by a modification of Bradford[4] using NIST traceable bovine serum albumin as the standard.

Purity

When examined on 4-12% SDS-PAGE gels, this protein migrates as a single major band with an apparent molecular weight of approximately 90,000 daltons. Densitometric analysis estimates the purity of the product as ≥ 90%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is < 2 EU/mg.

Activity

LF is assessed for specific activity in units/mg protein, using MAPKKide®, Product #530, which is a synthetic FRET peptide containing a single cleavage site for LF. A standard curve generated from MAPKKide® Unquenched Calibration Peptide for #530, Product #539, is used to convert relative fluorescence units (RFU) to µmoles of cleaved substrate. One unit of lethal factor will catalyze the release of 1.0 µmole of cleaved MAPKKide® per minute at 37°C in 20 mM HEPES, pH 8.2. The specific activity of this lot of lethal factor is 0.3 units/mg.

LF is assessed for cytotoxicity in the presence of 1 µg/mL PA using J774A.1 cells. When J774A.1 cells are treated with LF alone, no toxicity was seen at 1 µg/mL (11 nM). The effective concentration 50% (EC_{50}) of this LF lot meets specifications.

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Tissue Culture Applications

For tissue culture applications, medium containing glutamine must be fresh. Ammonium ions are released when glutamine breaks down and may prevent acidification of the endosome thereby inhibiting translocation of LF or Edema Factor (EF) into the cytosol.[5] A stable form of glutamine must be used.[6,7]

Handling

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including lab coat, gloves, and safety glasses.

This product is intended for research purposes by qualified personnel. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.

References

1. www.expasy.ch/tools/protparam-doc.html
2. Edelhoch, H. (1967) *Biochemistry*, **6**, 1948-1954.
3. Pace, C.N., Vajdos, F., Fee, L., Grimsley, G. and Gray, T. (1995) *Protein Sci.*. **4**, 2411-2423.
4. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248-254.
5. Stephen Little, Personal Communication.
6. GlutaMAX™ by Invitrogen/Gibco, www.invitrogen.com
7. Ala-Gln by Sigma, www.sigmaaldrich.com

Quality Assurance: _____ Date: 14 OCT 2021