



# LIST BIOLOGICAL LABORATORIES, INC.

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## CERTIFICATE OF ANALYSIS ANTHRAX LETHAL FACTOR (LF), RECOMBINANT from *Bacillus anthracis* Lot #1722B11B

### **Contents**

Each vial, when reconstituted with 0.5 ml of water, contains 1 mg of lethal factor from *Bacillus anthracis* in 5 mM HEPES, 50 mM NaCl, pH 7.5. Read the following recommendations prior to reconstituting this material. **Handle the product gently; do not vortex.**

### **Recommended Reconstitution and Storage of Anthrax Proteins**

Anthrax toxin proteins, when reconstituted with water, may be stored at 2-8°C and used successfully within a few hours. However, over longer periods of time, there will be a decline in the enzymatic activity of LF *in vitro* and the activity of the protective antigen (PA) + LF complex in living cells. If it is necessary to store this material, reconstitute at a concentration of 1 mg/ml.<sup>1</sup> Reconstitution with 1 mg/ml BSA will enhance stability and recovery.

It is further recommended that the solution be aliquoted and frozen at either -20°C or -80°C. Avoid repeated freeze-thaw cycles. After the protein has been reconstituted as described above, cold glycerol may be added to 50% if a liquid is desired at freezer temperatures. Storage of material reconstituted with 1 mg/ml BSA at 2-8°C for a period of two weeks may be acceptable for some applications.

### **Packaging/Storage**

This product is packaged aseptically, lyophilized and sealed under vacuum. Store at 2-8°C prior to reconstitution.

### **Concentration**

Protein concentration was determined by a modification of Bradford,<sup>2</sup> using bovine serum albumin as the standard.

### **Purity**

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

The endotoxin content, determined using a kinetic chromogenic LAL assay, is 1.6 EU/mg.

(continued)

### Activity

LF is assessed for specific activity in units/mg protein, using MAPKKide<sup>®</sup>, product #530, which is a synthetic FRET peptide containing a single cleavage site for LF. A standard curve generated from MAPKKide<sup>®</sup> Unquenched Calibration Peptide for 530, product #539, is used to convert relative fluorescence units (RFU) to  $\mu$ moles of cleaved substrate. One unit of lethal factor will catalyze the release of 1.0  $\mu$ mole of cleaved MAPKKide<sup>®</sup> per minute at 37°C in 20 mM HEPES, pH 8.2. The specific activity of this lot of lethal factor is 0.178 units/mg.

LF is assessed for cytotoxicity in the presence of 1  $\mu$ g/ml PA using J774A.1 cells. When J774A.1 cells are treated with LF alone, no toxicity is seen at 1  $\mu$ g/ml (11 nM). The effective concentration 50% (EC<sub>50</sub>) of this LF lot meets specifications.

### 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.<sup>3</sup> A stable form of glutamine must be used.<sup>4,5</sup>

### Handling

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.

This product is intended for research purposes only. 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 USE IN HUMANS.**

### References

1. Leppla, S.H. (1988) *Meth. Enz.* **165**, 103-116.
2. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248-254.
3. Stephen Little, personal communication.
4. Glutamax by Invitrogen/Gibco, [www.invitrogen.com](http://www.invitrogen.com)
5. Ala-Gln by Sigma, [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

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