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CERTIFICATE OF ANALYSIS  
ANTHRAX EDEMA FACTOR (EF), RECOMBINANT  
Mutant S447N from *Bacillus anthracis*  
Lot #1732A1

Contents:

Each vial contains 0.5 mg of edema factor from *Bacillus anthracis*. When reconstituted with 0.5 ml of sterile purified water, the concentration of buffer is 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 may be reconstituted in sterile purified water, stored at 2-8°C and used successfully within a few hours. However, over longer periods of time, there may be a decline in the enzymatic activity of EF. If it is necessary to store this material, reconstitute it 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 is aliquoted and frozen at either -20°C or -70°C. Avoid repeated freeze-thaw cycles. After the protein has been reconstituted as described above, glycerol may be added to 50% if a liquid is desired at freezer temperatures.

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 absorbance at 280 nm using an extinction coefficient of 0.874 for 1 mg/ml.<sup>2</sup>

Purity:

When examined on 10% polyacrylamide gels in the presence of SDS, this preparation migrates as a single major band with an apparent molecular weight of 89,000 daltons.

The endotoxin content, determined using a kinetic chromagenic LAL assay, is 153.5 EU/mg.

Activity:

The enzymatic activity of edema factor is assessed in a cell free system by measuring cAMP formation from ATP.<sup>3</sup> The activity of this EF lot is 227 µM cAMP/min/mg protein.

(continued)

The potency of EF to raise cAMP levels in Chinese hamster ovary (CHO) cells is tested in the presence of 1 µg/ml PA by the method of Kumar, *et al.*<sup>4</sup> The effective concentration 50% (EC<sub>50</sub>) of this EF lot is 10.1 µM cAMP/mg CHO cell protein/mg EF protein.

#### Tissue Culture Application:

For tissue culture applications, medium containing glutamine must be fresh. Ammonium ion is released when glutamine breaks down, and may prevent acidification of the endosome thereby inhibiting translocation of LF or EF into the cytosol.<sup>5</sup> A stable form of glutamine may be used.<sup>6,7</sup>

#### Handling

Good laboratory technique should be employed in the safe handling of this product. This requires observing the following practices:

1. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.
2. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.
3. Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.
4. This product is intended for research purposes by qualified personnel 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 HUMAN USE.**

#### Reference:

1. Leppla, S.H. (1988) *Meth. Enz.* **165**, 103-116.
2. Leppla, S.H. (1991) *Meth. Enz.* **195**, 153-168.
3. Hewlett, E.L., *et al.* (1991) *J. Biol. Chem.* **266**, 17503-17508.
4. Kumar, P., Ahuja, N., and Bhatnagar, R. (2002) *Infection and Immunity* **70(9)**, 4997-5007.
5. Stephen Little personal communication.
6. Glutamax by Invitrogen/Gibco, [www.invitrogen.com](http://www.invitrogen.com)
7. Ala-Gln by Sigma, [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

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