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Product #172B

CERTIFICATE OF ANALYSIS
ANTHRAX LETHAL FACTOR (LF), RECOMBINANT
from *Bacillus anthracis*
Lot #1722B10B

Contents:

Each vial contains 1.0 mg of lethal factor of *Bacillus anthracis*. When reconstituted with 1.0 ml of sterile distilled 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 distilled water, stored at 4°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* or the activity of the PA-LF complex in living cells. If it is necessary to store this material, reconstitute it at a concentration of 1 mg/ml.[1] 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. Storage of material reconstituted in BSA at 4°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 4°C prior to reconstitution.

Concentration:

Protein concentration was determined by modification of the method of Bradford,[2] using bovine serum albumin as the standard.

Purity:

When examined on 4-20% polyacrylamide gels in the presence of SDS, this preparation migrates as a band representing 91.5% of the protein with an apparent molecular weight of 90,000 daltons.

The endotoxin content, determined using a kinetic chromagenic LAL assay, is 1.06 EU/mg.

Activity:

LF is assessed for specific activity in units/mg protein, using MAPKKide™, a synthetic peptide containing a single cleavage site for LF. One unit is defined as the amount of lethal factor needed to 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 0.333 units/mg. This activity is 1.3 times the activity of the control lot of lethal factor.

(continued)

Activity:

LF is assessed for cytotoxicity in the presence of 1 µg/ml PA using J774A1 cells. Cell viability is measured using the Cell Counting Kit 8 (CCK-8) from Dojindo Molecular Technologies, Inc. When J774A.1 cells are treated with LF alone, no toxicity is seen at 1 µg/ml (12 nM). The effective concentration 50% (EC_{50}) of this LF lot meets specifications.

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.[3] A stable form of glutamine may be used.[4,5]

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. Bradford, M.M. (1976) *Anal. Biochem.* **72,** 248-254.

3. Stephen Little personal communication.

4. Glutamax by Invitrogen/Gibco, www.invitrogen.com

5. Ala-Gln by Sigma, www.sigmaaldrich.com

Approved: CT Date: 1/08/08 Approved: KA Date: 1/8/08