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Product #176B

CERTIFICATE OF ANALYSIS
RECOMBINANT
ANTHRAX MUTANT LETHAL FACTOR (LF)
from *Bacillus anthracis*
Lot #1761AA

Contents:

Each vial contains 1.0 mg of mutant lethal factor of *Bacillus anthracis*. This LF mutant strain has no enzymatic activity. 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. If it is necessary to store this material, reconstitute it at a concentration of 1.0 mg/ml.¹ Reconstitution with 1.0 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.

Concentration:

Protein concentration was determined by absorbance at 280 nm using an extinction coefficient of 0.812 for 1.0 mg/ml.²

Activity:

In immunodiffusion studies, anthrax mutant lethal factor shows similar immunoprecipitation as anthrax lethal factor when reacted against a specific antiserum to anthrax lethal factor. LF mutant does not cleave the synthetic peptide substrate MAPKKide™. No cytotoxicity was detected with 1 µg/ml (11 nM) LF mutant in the presence of 1 µg/ml (12 nM) PA when tested on J774.1A cells.

Purity:

When examined on 10% polyacrylamide gels in the presence of SDS, this preparation migrates as two major bands with an apparent molecular weight of 88,000 and 90,000 daltons.

(continued)

Packaging/Storage:

This product is packaged aseptically, lyophilized and sealed under vacuum. Store at 4°C prior to reconstitution.

Tissue Culture Application:

For tissue culture applications, medium containing glutamine must be fresh. Ammonium ion released when glutamine breaks down 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.

References:

1. Leppla, S.H. (1988) *Meth. Enz.* **165**, 103-116.
2. <http://www.expasy.ch/tools/protparam.html>
3. Stephen Little personal communication
4. Glutamax by Invitrogen/Gibco, www.invitrogen.com
5. Ala-Gln by Sigma, www.sigmaaldrich.com

Approved: cl

Date: 6/29/07

Approved: DN

Date: 6/29/07