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Product #177A

CERTIFICATE OF ANALYSIS
ANTHRAX PROTECTIVE ANTIGEN, Fragment (PA 20)
17-20 kDa Cleavage Product from *Bacillus anthracis*
Lot #1771A1

Contents:

Each vial contains 50 µg of the 17-20 kDa fragment produced when protective antigen from *Bacillus anthracis* is cleaved with trypsin. This fragment has been purified to greater than 98% purity as assessed by SDS page gels. When reconstituted with 0.2 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. If it is necessary to store this material, reconstitute it at a concentration of 1 mg/ml.[1] Reconstitution with 1 mg/ml BSA may enhance stability and recovery.

It is further recommended that the solution is aliquoted and frozen at either -20° or -70°C. 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 2-8°C for a period of two weeks may be acceptable for some applications.

Concentration:

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

Purity:

When examined on 18% polyacrylamide gels in the presence of SDS, this preparation migrates as a single major band with an apparent molecular weight of 20,000 daltons. Several slower migrating minor components are also apparent, and may represent fragments of the protective antigen.[3,4]

The molecular weight determined by electrospray ionization mass spectrometry is 17,160 daltons.

Packaging/Storage:

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

(continued)

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.[5] A stable form of glutamine may be used.[6,7]

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. Singh, Y., Klimpel, K.R., Goel, S., Swain, P.K. and Leppla, S.H. (1999) *Infect. Immun.* **67,** 1853-1859.

4. Mogridge, J., Cunningham, K., Lacy, D.B., Mourez, M., Collier, R.J. (2002) *Proc. Natl. Acad. Sci.* **99,** 7045-7048.

5. Stephen Little personal communication

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

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

Approved: CL Date: 7/22/09 Approved: KRC Date: 7/22/09