LIST BIOLOGICAL LABORATORIES, INC.

540 DIVISION STREET ▪ CAMPBELL ▪ CALIFORNIA 95008-6906 ▪ USA
408-866-6363 ▪ 800-726-3213 ▪ FAX 408-866-6364 ▪ EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Product #175

CERTIFICATE OF ANALYSIS
PROTECTIVE ANTIGEN 63 FITC CONJUGATE
LOT #1751A

Preparation:

FITC is prepared by conjugating anthrax protective antigen to fluorescein isothiocyanate, isomer I (FITC) by a modification of the method of Wood *et al.*[1] PA has been trypsin activated and the PA63 conjugate to FITC has been purified.

Contents:

When reconstituted with 0.2 ml of sterile water, each vial contains 50 µg of protein in 10 mM bis-Tris propane, pH 8.5 with 1.25% trehalose. **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 may be a decline in the PA binding. If it is necessary to store this material, reconstitute it at a concentration of 1 mg/ml.[2] 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 4°C for a period of two weeks may be acceptable for some applications.

Binding Ratio:

This lot contains 21 µg of FITC bound per mg of PA 63 fragment of anthrax protective antigen, assuming an extinction coefficient at 493 nm of 85,200 $M^{-1}cm^{-1}$ for FITC.[3]

Purity:

When examined on 4-15% polyacrylamide gels in the presence of SDS, this preparation migrates as a single major band with an apparent molecular weight of 63,000 daltons. Several faster migrating minor components are also apparent, and may represent fragments of the protective antigen.[4,5]

Packaging/Storage:

This product is provided as a lyophilized powder that has been sealed under vacuum. Store at 4°C prior to and following reconstitution. **This product is light sensitive.**

(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.[6] A stable form of glutamine may be used.[7,8]

Handling:

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

1. **This product is to be used by skilled personnel under the direction of a principal investigator in a laboratory setting only.**

2. **Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.**

3. **Never remove the stopper prior to reconstitution and never work with the product in the powdered form. Always reconstitute it first.**

4. **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.**

5. **Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.**

6. **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. Wood, B.T., Thompson, S.H. and Goldstein, G. (1965) *J. Immun.* **95**, 225-229.

2. Leppla, S.H. (1988) *Meth. Enz.* **165**, 103-116.

3. Moller, G., Gronowitz, E., Persson, U., Coutinho, A., Moller, E., Hammarstrom, L., and Smith, E. (1976) *J. Exp. Med.* **143**, 1429-1438.

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

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

6. Stephen Little personal communication.

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

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

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