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Product #171E
Lot #17121A1
Release Date: February 2020

CERTIFICATE OF ANALYSIS
ANTHRAX PROTECTIVE ANTIGEN (PA)
RECOMBINANT from *Bacillus anthracis*
Lot #17121A1

Contents

Each vial contains 0.5 mg of Protective Antigen from *Bacillus anthracis*. When reconstituted to 0.5 ml with water, the buffer is 0.005 M HEPES, 0.05 M NaCl, pH 7.5. **Handle the product gently; do not vortex.**

Packaging/Storage

Anthrax toxin proteins, when reconstituted with water, should be used within a few hours.

Packaging/Storage

This product is provided as an aseptically packaged lyophilized powder, 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 0.97 for a 1 mg/ml solution. This value is calculated by ProtParam¹ using an algorithm based on the Edelhoch² method with modifications described in Pace et al³.

Purity

When examined on 4 – 12% SDS-PAGE gels, this protein migrates as a single major band with an apparent molecular weight of approximately 83,000 daltons. Densitometric analysis estimates the purity as >95%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is < 1 EU/mg.

Activation

In certain systems, this product may require trypsinization to generate the active C-terminal 63,000 dalton fragment.⁴ Purified nicked Protective Antigen, product #174, is available.

Cytotoxic Activity

PA is assessed for cytotoxicity in the presence of 1 µg/mL LF using J774A.1 cells. When J774A.1 cells are treated with PA alone, no cytotoxicity was seen at 1 µg/mL. The effective concentration 50% (EC₅₀) of this PA lot meets specifications.

(continued)

Tissue Culture Application

For tissue culture applications, medium containing glutamine must be fresh. Ammonium ions are released when glutamine breaks down, and may prevent acidification of the endosome thereby inhibiting translocation of LF or Edema Factor (EF) into the cytosol.⁵ A stable form of glutamine must be used.^{6,7}

Handling

Good laboratory technique should be employed in the safe handling of this product; refer to the SDS. Wear appropriate laboratory attire including lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.

This product is intended for research purposes by qualified personnel. 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. www.expasy.ch/tools/protparam-doc.html
2. Edelhoch, H. (1967) *Biochemistry*, **6**, 1948-1954.
3. Pace, C.N., Vajdos, F., Fee, L., Grimsley, G. and Gray, T. (1995) *Protein Sci.* **4**, 2411-2423.
4. Bhatnagar, R., Singh, Y., Leppla, S.H., Friedlander, A.M. (1989) *Infect. Immun.* **57**, 2107-2114.
5. Stephen Little, Bacteriology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, 21702, personal communication, 2003.
6. GlutaMAX™ by Invitrogen/Gibco, www.invitrogen.com
7. Ala-Gln by Sigma, www.sigmaaldrich.com

QA/QC: WAT Date: 03 NOV 2020