

PH CHANGE

CERTIFICATE OF ANALYSIS
ANTHRAX PROTECTIVE ANTIGEN, ACTIVATED (PA 63)
from *Bacillus anthracis*
Lot #17410A1

Contents

Activated protective antigen (PA 63) has been prepared by trypsinization of protective antigen followed by removal of the 20 kDa cleavage product. PA 63 forms an oligomer and binds to both lethal factor (LF) and edema factor (EF).

Each vial, when reconstituted with 0.5 ml water, contains 0.5 mg of activated protective antigen from *Bacillus anthracis* in 10 mM bis-Tris propane, 0.1 M NaCl, pH 9.0 with 1.25% trehalose. 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, when reconstituted in water, may be 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 will enhance stability and recovery.

It is further recommended that the solution be aliquoted and frozen at either -20° or -80°C. Avoid repeated freeze-thaw cycles. After the protein has been reconstituted as described above, cold glycerol may be added to 50% if a liquid is desired at freezer temperatures. Storage of material reconstituted with 1 mg/ml BSA at 2-8°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 2-8°C prior to reconstitution.

Concentration

Protein concentration was determined by a modification of Bradford², using bovine serum albumin as the standard.

Purity

When examined on 4-12% polyacrylamide gels in the presence of SDS, this protein migrates as a single major band with an apparent molecular weight of 63,000 daltons. Densitometric analysis estimates the purity of the product as >95%.

The endotoxin content, determined using a kinetic chromagenic LAL assay, is 1.16 EU/mg.

(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 EF into the cytosol.³ A stable form of glutamine must be used.^{4,5}

Handling

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

This product is intended for research purposes 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. 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