

CERTIFICATE OF ANALYSIS  
BINARY TOXIN from *C. difficile*, A SUBUNIT  
Lot #1573A1

**Contents**

Each vial contains 20 µg of Binary toxin, A subunit. When reconstituted with 0.1 ml of water, the concentration of the buffer is 20 mM HEPES, pH 7.4 + 1.25% lactose. **Handle the product gently; do not vortex.**

The protein was recombinantly expressed in *E. coli* and purified using affinity chromatography. The affinity tag has subsequently been cleaved from the protein prior to quantitation and packaging.

**Molecular Weight**

The Binary toxin, A subunit contains amino acids 44 - 463 of the full length, omitting the leader sequence. Five residual amino acids from the affinity tag at the N - terminus give a total length of 425 amino acids. The molecular weight of the protein is calculated to be 48,676 Da based on the amino acid sequence.

**Concentration**

The protein concentration was determined by absorbance at 280 nm using  $Abs(0.1\%) = 1.217$ . This value is calculated by ProtParam<sup>1</sup> using an algorithm based on the Edelhoch<sup>2</sup> method with modifications described in Pace et al<sup>3</sup>.

**Purity**

When examined on a 4-12% SDS-polyacrylamide gel under reducing conditions, this product migrates as a single major band with an apparent molecular weight of approximately 50,000 Da. Densitometric analysis estimates the purity of the product as >95%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is approximately 3,000 EU/mg.

**Activity**

The Binary toxin, A subunit has been tested in an *in vitro* ADP-ribosylation assay. The activity was measured in 30 mM sodium phosphate, pH 7.0, containing 7 mM β-NAD, 20 mM dithiothreitol and 30 mM agmatine as the ADP-ribosyl acceptor. The reaction is initiated by addition of the A subunit and incubation proceeds for 90 minutes at 30°C. The nicotinamide generated is monitored using reverse phase HPLC. The specific activity is 22 nmoles of nicotinamide/mg of toxin/min.

**Packaging and Storage**

This product is supplied as a lyophilized powder which has been stoppered under vacuum. Reconstitution of the powder should be done with a syringe through the rubber stopper to avoid any loss of material. Store lyophilized vials at 2-8°C. Once dissolved, aliquot and store the product at -20°C. Refrain from multiple freeze/thaw cycles.

(continued)

### Toxicity

The A subunit of the Binary toxin is non-toxic and unable to penetrate cells in the absence of the B subunit (binding and translocation domain). The expression and purification of the A subunit from a recombinant setting ensures that there is no possible contamination with the B subunit.

### Handling

This product is not known to be hazardous. 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 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. [www.expasy.ch/tools/protparam-doc.html](http://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.

Production: TC Date: 11/28/12 Management: NS Date: 11/14/12 QA/QC: EP Date: 11/14/12