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Product #141A  
Lot #1416A3  
Release Date: August 2017  
Recommended Retest Date: August 2020

CERTIFICATE OF ANALYSIS  
BOTULINUM NEUROTOXIN TYPE E, NICKED,  
from *Clostridium botulinum*  
Lot #1416A3

**Contents**

Each vial, when reconstituted with 0.1 ml of water, contains 10 µg of nicked botulinum neurotoxin type E in 20mM Sodium Citrate, 3mM Sodium Acetate, pH 6.0 with 24mM Trehalose and 1.25% Lactose. This product has been activated by nicking. **To ensure full recovery of toxin from the vial, include 1 mg/mL of BSA or 0.2% TWEEN 20 in the reconstitution buffer. Handle the product gently; mix by inversion, do not vortex. READ ALL HANDLING INFORMATION PRIOR TO RECONSTITUTION.**

**Concentration**

Protein concentration was determined by absorbance at 280 nm using an extinction coefficient of 1.16 for a 1 mg/ml solution. 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>.

NOTE: The concentration for previous lots of Product #141A was determined using a published extinction coefficient of 1.45.

**Purity**

When examined on 4 – 12% SDS-PAGE gels under non-reducing conditions, this product migrates as a single major band with an apparent molecular weight of approximately 150,000 daltons. Densitometric analysis estimates the purity of the product as ≥ 95%.

When examined on 4 – 12% SDS-PAGE in the presence of a reducing agent, the preparation migrates as two major bands corresponding to the heavy chain (100,000 daltons) and the light chain (50,000 daltons), respectively. The band corresponding to the intact toxin at 150,000 daltons is approximately 7% of the total toxin indicating 93% nicked toxin.

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

**Activity**

This nicked *C. botulinum* neurotoxin type E has been tested for activity in an endopeptidase assay using SNAP-25 (Product #500A) as the substrate. The neurotoxin, at 20 nM, cleaves >80% of the 5 µM substrate in 15 minutes at 37°C, with complete cleavage in 60 minutes.

(continued)

### Toxicity

This product has been activated by nicking. Toxicity testing has not been done on this lot. Botulinum toxin is the most deadly bacterial toxin known to man. The minimum lethal dose (LD<sub>100</sub>) of *C. botulinum* neurotoxin type E in mice is estimated at 1.1 ng/kg, i.p. Humans are said to be at least as sensitive as mice.<sup>4</sup> Consult the SDS for further information.

### Packaging/Storage

This product is packaged aseptically, lyophilized and sealed under vacuum. Store at 2 – 8°C.

### Handling

Good laboratory technique should be employed in the safe handling of the neurotoxin. Wear appropriate attire including lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material. Persons handling this product should consult the current version of the Biosafety in Microbiological and Biomedical Laboratories.<sup>5</sup>

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. Edelhoich, 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. Gill D.M. (1982) *Microbiol. Rev.* **46**, 86-94.
5. Biosafety in Microbiological and Biomedical Laboratories. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.

Production: KO Date: 8-16-17 Management: NS Date: 8-16-17 QA/QC: KAP Date: Aug 16, 2017