

CERTIFICATE OF ANALYSIS  
BOTULINUM NEUROTOXIN TYPE E, NICKED,  
from *Clostridium botulinum*  
Lot #1413A1

### Contents

Each 10 µg vial of nicked botulinum neurotoxin type E, when reconstituted with 110 µl of water, contains 10 µg of protein in 20 mM sodium citrate, pH 6.0 with 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 1.45 for a 1 mg/ml solution.

### Gel Electrophoresis

When examined on 4-12% SDS-polyacrylamide gels, this product migrates as a single major band with an apparent molecular weight of approximately 150,000 daltons. 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 8% of the total toxin indicating 92% nicked toxin.

### Purity

The product migrates as one major band under non-reducing conditions, when examined by SDS polyacrylamide gel electrophoresis. The purity is  $\geq 90\%$ . The endotoxin content, determined using a kinetic chromagenic LAL assay, is 65.3 EU/mg. The absorbance ratio ( $OD_{260}/OD_{280}$ ) of this preparation is 0.58.

### 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.

### 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 ( $LD_{100}$ ) of *Clostridium botulinum* type E neurotoxin in mice is estimated at 1.1 ng/kg, i.p. Humans are said to be at least as sensitive as mice.<sup>1</sup> Consult the MSDS for further information.

(continued)

### Storage

This product is supplied as a lyophilized powder which has been stoppered under vacuum. Store at 2-8°C.

### Handling

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

1. Persons handling this product and contaminated glassware should consult the current version of the Biosafety in Microbiological and Biomedical Laboratories.<sup>2</sup>
2. This product is to be used by skilled personnel under the direction of a principal investigator in an appropriate laboratory.
3. Wear appropriate attire, i.e., labcoat, eye protection and gloves. Nitrile gloves are recommended when handling lyophilized material.
4. Because this product is stoppered under vacuum, remove the stopper very slowly in a biological safety cabinet prior to reconstitution. Never work with the product in the powdered form. Always reconstitute it first.
5. 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.
6. Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.
7. 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 USE IN HUMANS.**

### References

1. Gill, D.M. (1982) *Microbiol. Rev.* **46**, 86-94.
2. 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: WAC Date: 4/20/12 Management: NS Date: 4/20/12 QA/QC: CHP Date: 4/23/12