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CERTIFICATE OF ANALYSIS
BOTULINUM NEUROTOXIN TYPE D, NICKED, RECOMBINANT
Lot #1461A1

Contents

Each vial contains 10 µg of recombinant Botulinum Neurotoxin Type D (rBoNT/D). When reconstituted with 100 µl of water the protein is in PBS, pH 7.4. **To ensure full recovery of toxin from the vial, include 1 mg/ml 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 estimated protein coefficient of 1.32 for a 1 mg/ml solution. This value is calculated by ProtParam¹ using an algorithm based on Edelhoch² method with modifications described in Pace et al.³

Analysis

When examined on 4-12% SDS-PAGE, this protein 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 bands with apparent molecular weights of 100,000 and 50,000 daltons. Densitometric analysis indicates the purity is >95%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is ~ 7 EU/mg.

Activity

The activity of a 20 nM solution of rBoNT/D, as measured by the percent cleavage of GST Synaptobrevin-2 (Product #510) in 0.02M Tris, 0.05 M NaCl, pH 8.0 with 0.02 M ZnCl₂ and 5 mM DTT, is 85% in 60 minutes.

Toxicity

Toxicity testing has not been done on this lot. Botulinum toxin is the most deadly bacterial toxin known to man. The minimum lethal dose (LD₁₀₀) in mice is estimated at 1.2 ng/kg, i.p. Humans are said to be at least as sensitive as mice.⁴ Consult the MSDS for further information.

Storage

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

(continued)

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.⁵
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. Never work with the product in the powdered form. Always reconstitute it as directed by the principal investigator. Note: This product is stoppered under vacuum.
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. 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. 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: 8-25-2014

Management: NS Date: 8-25-14

QA/QC: hsp Date: 8/25/14