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CERTIFICATE OF ANALYSIS  
BOTULINUM NEUROTOXIN TYPE D LIGHT CHAIN, Recombinant  
Lot #6301A1

Contents:

Each vial of recombinant light chain from botulinum neurotoxin type D contains 10 µg of lyophilized protein. When reconstituted with 33 µl of sterile distilled water, each vial contains 10 µg of light chain in 20 mM HEPES, pH 7.4 + 1.25% lactose. The pH 7.4 HEPES buffer was obtained by titrating the free acid form of HEPES with the potassium salt form of HEPES. This minimizes the sodium and chloride concentrations present with the toxin. In order to ensure stability during storage of the protein 0.05% TWEEN-20 or 1 mg/ml BSA must be included in your reconstitution buffer.

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

Molecular Weight:

The light chain D fragment contains amino acids 1 - 436 of the full length botulinum neurotoxin type D. It also contains eight residual amino acids from the affinity tag at the N-terminus to give a total length of 444 amino acids. The molecular weight of the protein is approximately 50600 daltons.

Concentration:

Protein concentration was determined by a modification of the Bradford<sup>1</sup> method using bovine serum albumin as a standard.

Gel Electrophoresis:

When examined on 12% SDS-polyacrylamide gels, this product migrates as a single major band with an apparent molecular weight of approximately 150,000 daltons. The protein appears to be greater than 90% pure based on densitometry.

Activity:

The recombinant light chain from botulinum neurotoxin type D has been tested for activity in an endopeptidase assay. 20 nM light chain D was able to cleave 5 µM His<sub>6</sub>-Synaptobrevin at 37°C in 20 mM Tris-HCl, pH 8.0 and 50 mM NaCl. Full cleavage was achieved within 30 minutes of initiation of the reaction.

(continued)

### 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 syringe through the rubber stopper to avoid any loss of material. Store lyophilized vials at 4°C. Once dissolved, aliquot and store at -20°C. Refrain from multiple freeze/thaw cycles.

### Toxicity:

The light chain of Botulinum neurotoxin is non-toxic and unable to penetrate cells in the absence of the heavy chain. The expression and purification of light chain from a recombinant setting ensures there is no possible contamination with heavy chain or full length intact toxin.

### Handling:

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

1. Wear appropriate laboratory attire including a labcoat, gloves and safety glasses.
2. 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.
3. Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.
4. This product is intended for research purposes by qualified personnel 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. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248-254.

Approved: NS Date: 7-5-06 Approved: TC Date: 7/5/06