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Product # 620A

# CERTIFICATE OF ANALYSIS RECOMBINANT LIGHT CHAIN from BOTULINUM NEUROTOXIN TYPE B Lot # 62015A1

### Contents:

Each vial contains 10 µg of light chain from botulinum neurotoxin type B. When reconstituted with 0.1 ml of water, the concentration of the buffer is 20 mM HEPES, pH 7.4 + 1.25% lactose. In order to ensure stability during storage of the protein, 0.05% TWEEN-20 or 1 mg/ml BSA must be included in the reconstitution buffer. **Handle the product gently; do not vortex.** 

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

# Molecular Weight:

The light chain type B fragment contains amino acids 1 - 436 of the full length botulinum neurotoxin type B. 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 has been determined to be 51210 Da based on electorspray-MS analysis.

# Concentration:

Protein concentration was determined by absorbance at 280 nm using Abs (0.1%) = 0.80. 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>.

#### Gel Electrophoresis:

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.

#### Activity:

The protein has been tested for activity in a FRET endopeptidase assay. Significant cleavage of the fluorogenic peptide substrate VAMPtide® (Product #540) was seen with 2.5, 5, and 10 nM enzyme.

# Packaging and Storage:

This product is supplied as a lyophilized powder which has been stoppered under vacuum. Store lyophilized vials at 2-8°C. Once dissolved, aliquot and store the product at -20°C. Refrain from multiple freeze/thaw cycles.

#### Toxicity:

The light chains of the botulinum neurotoxins are non-toxic and unable to penetrate cells in the absence of the heavy chain (binding and translocation domains). 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.

(continued)

# Handling:

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

- 1. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.
- 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 HUMAN USE.

# 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.

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