



540 DIVISION STREET ■ CAMPBELL ■ CALIFORNIA 95008-6906 ■ USA
408-866-6363 ■ 800-726-3213 ■ FAX 408-866-6364 ■ EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Product # 611A

CERTIFICATE OF ANALYSIS
RECOMBINANT LIGHT CHAIN FROM BOTULINUM NEUROTOXIN TYPE A, GST FUSION
Lot # 6112A1

Contents

Each vial of Botulinum Neurotoxin Type A, light chain (BoNT/A LC) GST fusion protein, when reconstituted with 100 μ l water, contains 15 μ g of protein 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 protein. In order to ensure stability during storage and recovery of the protein, 0.05% TWEEN-20 or 1 mg/ml BSA must be included in the reconstitution buffer.

The protein was recombinantly expressed in *E. coli* and purified using affinity chromatography. The affinity tag has been retained.

Molecular Weight

The BoNT/A LC fragment contains amino acids 1 - 429 of the full length Botulinum Neurotoxin Type A. This molecule also contains the GST affinity tag at the N - terminus to give a total length of 663 amino acids. The molecular weight of the protein is calculated to be 76,396 Da based on amino acid sequence.

Concentration

Protein concentration was determined by absorbance at 280nm using Abs (0.1%) = 1.173. This value is calculated by ProtParam¹ using an algorithm based on the Edelhoch² method with modifications described in Pace et al³.

Purity

When examined on 4-12% SDS-polyacrylamide gels, this product migrates as a single major band with an apparent molecular weight of approximately 75,000 Da. The protein purity is > 85% based on densitometric analysis.

Activity

The BoNT/A LC fusion has been tested for enzymatic activity in a FRET based assay using 5 μ M SNAPtide[®] (Product #520), a FRET substrate specific for BoNT/A, and 5 nM BoNT/A LC. The substrate is readily digested at 37°C in 50 mM HEPES, pH 7.4 containing 0.05% Tween-20.

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 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

This product is not known to be hazardous. Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.

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
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.

Production: TE Date: 7/24/14 Management: NG Date: 7/24/14 QA/QC: GP Date: 7/24/14