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Product # 611A

CERTIFICATE OF ANALYSIS
BOTULINUM NEUROTOXIN TYPE A LIGHT CHAIN, GST Fusion
Lot # 6111A1

Contents:

Each vial of light chain from Botulinum neurotoxin type A contains 15 μ g of protein lyophilized with 180 μ l of 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 and recovery 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 and ion exchange chromatography. The GST affinity tag has been retained on this protein.

Molecular Weight:

The light chain A fragment contains amino acids 1 - 429 of the full length Botulinum Neurotoxin Type A for a molecular weight of approximately 50, 000 Da. The GST tag is 26, 000 Da. The total molecular weight of the fusion protein based on sequence is 76,389 Da.

Concentration:

Protein concentration was determined by a modification of the Bradford¹ method using bovine serum albumin as a standard.

Gel Electrophoresis:

When examined on 12% SDS-polyacrylamide gels, this product migrates as a major band with an apparent molecular weight of approximately 76,000 Da. The protein is 80% pure based on densitometry analysis. Faint bands with the approximate size of the light chain and GST proteins can also be seen.

Activity:

The recombinant Light Chain- GST fusion has been tested for activity in a FRET based assay. A standard curve was generated using SNAPtide[®] Unquenched Calibration Peptide (Prod. # 529). When using our SNAPtide[®] substrate (Product #520), cleavage can be easily detected at 5 nanomolar concentration of protein.

(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 requires observing the following practices:

- 1. Wear appropriate laboratory attire including a lab coat, 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 HUMAN USE.

References:

1. Bradford, M.M. (1976) *Anal. Biochem.* 72, 248-254.

Approved: WS Date: 1/14/08

Approved: TC Date: 1/14/08