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Product # 622A

CERTIFICATE OF ANALYSIS  
Botulinum Neurotoxin Type B Heavy Chain Binding Domain  
Lot # 6221A1

Contents:

Each vial of recombinant Botulinum Neurotoxin Type B Heavy Chain Binding Domain (HccB) contains 50.0  $\mu$ g of lyophilized protein. When reconstituted with 500  $\mu$ l of sterile distilled water, each vial contains 50.0  $\mu$ g of HccB in 20 mM HEPES, pH 7.4 + 1.25% lactose. The protein was recombinantly expressed in *E. coli* and purified using affinity and anion exchange chromatography. The affinity tag has subsequently been cleaved off of the protein prior to quantitation and packaging.

Molecular Weight:

HccB is 447 amino acids in length. This product contains amino acids 858-1291 of the Botulinum Neurotoxin Type B Okra Strain. There are also 13 residual amino acids on the N- terminus from the affinity tag. The molecular weight of the protein is approximately 53 kD.

Concentration:

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

Gel Electrophoresis:

This product migrates as a single major band on 4-12% SDS polyacrylamide gels with an apparent molecular weight of 50 kD. The protein is greater than 95% pure based on densitometry.

Activity:

HccB is highly reactive to Anti-Botulinum Type B antibodies in a Western Blot. Botulinum Neurotoxin Type B Heavy Chain Binding Domain was tested in an ELISA assay using GT1b and the receptor domain of Synaptotagmin II. The midpoint of the binding curve of HccB to Synaptotagmin II was 5.7 ng. The midpoint of the binding curve of HccB to GT1b was 11.4 ng.

(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:

HccB is only a fragment of the Botulinum toxin and is thus non-toxic.

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: TC Date: 2/10/10 Approved: NS Date: 2/10/10