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Product # 622A

# CERTIFICATE OF ANALYSIS HEAVY CHAIN BINDING DOMAIN from BOTULINUM NEUROTOXIN TYPE B Lot # 6222A1

### Contents

Each vial of recombinant Heavy Chain Binding Domain from Botulinum Neurotoxin Type B (HccB) contains 50  $\mu$ g of lyophilized protein. When reconstituted with 150  $\mu$ l of water, the protein is 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 absorbance at 280nm using Abs (0.1%) = 1.977. 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>.

#### Purity

When examined on 4-12% SDS-polyacrylamide gels, this product migrates as a single major band with an apparent molecular weight of approximately 50,000 Da. The protein purity is >95% based on densitometric analysis.

#### Activity

HccB (0.5  $\mu$ g) is readily detected in a Western Blot analysis using a 1:1000 dilution of Anti-Botulinum Neurotoxin Type B, Product #736. HccB was tested in an ELISA assay confirming that HccB binds to both GT1b and the receptor domain of Synaptotagmin II.

# 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**

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

#### 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.

QA/QC: <u>FPD</u> Date: <u>01|22|202|</u>

Made in U.S.A.

