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Product #612A

# CERTIFICATE OF ANALYSIS RECOMBINANT HEAVY CHAIN BINDING DOMAIN FROM BOTULINUM NEUROTOXIN TYPE A Lot #6123A1

### Contents

Each vial of recombinant Botulinum Neurotoxin Type A Heavy Chain Binding Domain (HccA) contains 50  $\mu$ g of lyophilized protein, which when reconstituted with 100  $\mu$ l of water, is in 20 mM HEPES, pH 7.4, 150mM NaCl + 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**

HccA is 436 amino acids in length. This product contains 10 residual amino acids from the GST tag and amino acids 872-1296 of the Botulinum Neurotoxin Type A Hall Strain. The molecular weight of the protein is approximately 51 kDa.

### Concentration

Protein concentration was determined by absorbance at 280nm using Abs (0.1%) = 1.777. This value is calculated by ProtParam¹ using an algorithm based on the Edelhoch² method with modifications described in Pace et al³.

#### **Gel Electrophoresis**

This product migrates as a single major band on 4-12% SDS polyacrylamide gels with an apparent molecular weight of 50 kDa. The protein is >95% pure under reducing conditions based on densitometric analysis.

## **Activity**

HccA is reactive to anti-Botulinum Type A antibodies, Product #730A, in a Western Blot. HccA was tested in an ELISA assay using plates coated with 2 µg of either GT1b or the receptor domain of SV2c, Product #690A. The binding to GT1b as well as to SV2c can be detected down to ~5 ng of HccA.

#### **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 protein at -20°C. Refrain from multiple freeze/thaw cycles.

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## **Toxicity**

HccA is only a fragment of Botulinum toxin and, as such, is a non toxic protein.

## **Handling**

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 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.

QA/QC: FPD Date: 22 Jan 2021

Made in U.S.A.

