

CERTIFICATE OF ANALYSIS
GST-Synaptobrevin-2 Recombinant Protein Substrate for *C. botulinum*
Neurotoxin Types B, D, and F
Lot # 5108A1

Contents

Each 100 µg vial of recombinant GST-Synaptobrevin-2, when reconstituted with 185 µl of water, contains 100 µg of protein in 20 mM HEPES, pH 7.4 + 1.25% lactose.

The protein was recombinantly expressed in *E. coli* and purified using affinity and ion exchange chromatography. The GST affinity tag has been retained on the protein.

Molecular Weight

GST-Synaptobrevin-2 is 331 amino acids in length. This product contains the GST affinity tag and 97 amino acids of the 116 amino acids of the Synaptobrevin-2 sequence. The final 19 amino acids of the native protein, containing the transmembrane domain, are not included in this preparation of Synaptobrevin-2. The molecular weight of the protein is 37,797 daltons based on analysis of the nucleic acid sequence.

Concentration

Protein concentration was determined by absorbance at 280nm using $Abs(0.1\%) = 1.471$. 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 migrated with an apparent molecular weight of approximately 38,000 Da. Densitometric analysis indicates that the protein purity is > 85%.

Activity

The recombinant light chain from botulinum neurotoxin Type D (BoNT/D LC) (Product #630A) has been used to test the GST-Synaptobrevin-2 in an endopeptidase assay. Twenty nanomolar BoNT/D LC was incubated with 5 µM GST-Synaptobrevin-2 in 20 mM Tris, pH 8.0, containing 50 mM NaCl and 5 mM DTT at 37°C. In 1 hour, 95% of the GST-Synaptobrevin-2 was shown to be cleaved using SDS PAGE analysis.

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

GST-Synaptobrevin-2 is not known to be toxic.

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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: TC Date: 7/11/12

Management: NS Date: 7/11/12

QA/QC: GP Date: 7/11/12