

**CERTIFICATE OF ANALYSIS  
SNAP-25, RECOMBINANT PROTEIN SUBSTRATE  
FOR *C. BOTULINUM* TYPES A AND E NEUROTOXIN  
Lot #5004A1**

**Contents**

Each vial contains 100 µg of lyophilized recombinant SNAP-25. When reconstituted with 100 µl of water, the protein is in 20 mM HEPES, pH 7.4 + 1.25% lactose. This 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**

SNAP-25 is 206 amino acids in length. This product also contains eight residual amino acids from the affinity tag at the N-terminus to give a total length of 214 amino acids. The calculated molecular weight of the protein is 24,120 daltons.

**Concentration**

Protein concentration was determined by absorbance at 280 nm using Abs (0.1%) = 0.3. 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-PAGE, this product migrates with an apparent molecular weight of approximately 25,000 Da. The protein purity is > 65% based on densitometric analysis.

**Activity**

SNAP-25 can be used as a substrate to assess the activity of Botulinum Neurotoxin Type A, Product #128 and #130, Botulinum Neurotoxin Type A light chain (LcA), Product #610A, Botulinum Neurotoxin Type E, Product #140 and #141, and Botulinum Neurotoxin Type E light chain (LcE), Product #635A. A 100 µl reaction of 5 µM SNAP-25 (12 µg) was completely cleaved within 15 minutes at 37°C in 20 mM Tris-HCl, pH 8.0 and 50 mM NaCl using LcE. Detection of the cleavage of SNAP-25 by LcE is readily apparent by running a sample of the digestion on a coomassie stained SDS-PAGE. The cleavage of SNAP-25 by Type A Botulinum Neurotoxin or light chain is not detectable on a gel, due to the small cleavage product, but can be visualized by performing a Western Blot with primary antibodies specific to Botulinum Type A cleaved SNAP-25 (Research and Diagnostic Antibodies, Inc., Las Vegas, NV).



540 Division Street, Campbell, California 95008-8908  
Tel. (408) 866-6363 Fax (408) 866-6364  
[www.listlabs.com](http://www.listlabs.com)

Product #500A  
Lot #5004A1

### 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

SNAP-25 is not known to be 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](http://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.

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