

CERTIFICATE OF ANALYSIS
SNAP-25 RECOMBINANT PROTEIN SUBSTRATE for
C. botulinum NEUROTOXINS TYPE A and E
Lot # 5003A1

Contents

Each vial of recombinant SNAP-25 contains 100 µg of lyophilized protein. When reconstituted with 265 µl of water, the resulting buffer is 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

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 molecular weight of the protein is approximately 24,120 daltons.

Concentration

Protein concentration was determined by a modification of the Bradford¹ method using bovine serum albumin as a standard.

Gel Electrophoresis

This product migrates as a single major band on 4-12% SDS-PAGE with an apparent molecular weight of 25,000 Da. The protein is 90% pure based on densitometry.

Activity

SNAP-25 can be used as a substrate to assess the enzymatic activity of Botulinum Neurotoxin Type A, product #130A, Botulinum Neurotoxin Type A light chain (LcA), product #610A, 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 one hour at 37°C in 20 mM Tris-HCl, pH 8.0 and 50 mM NaCl using 20 nM of either LcA or 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 LcA is not detectable on an SDS-PAGE, 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.).

(continued)

Product # 500A, Lot #5003A1

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 a syringe through the rubber stopper to avoid any loss of material. Store lyophilized vials at 2-8°C. Once dissolved, aliquot and store at -20°C. Refrain from multiple freeze/thaw cycles.

Toxicity

SNAP-25 is not known to be toxic.

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. Bradford, M.M. (1976) *Anal. Biochem.* 72, 248-254.

Production: KD Date: 12-1-15

Management: NS Date: 11/30/15

QA/QC: YLP Date: 11/30/15

Made in U.S.A. 