

CERTIFICATE OF ANALYSIS
VAMPtide® (PL 150, Pya/Nop)
Peptide Substrate for Botulinum Neurotoxin Type B
Lot #5421A1

Contents

Each vial of VAMPtide® (PL 150, Pya/Nop), a botulinum neurotoxin type B (BoNT/B) substrate, contains 200 nmoles of lyophilized peptide which is stoppered under vacuum. Store at -20°C, protected from light.

This peptide is an intramolecularly quenched fluorogenic substrate, modified on both sides of the cleavage site. A pyrenylalanine (Pya) is N-terminal and a p-nitrophenylalanine (Nop) is C-terminal to the cleavage site. This peptide is supplied by Pharmaleads, Paris, France and is described in the publication by Anne, C., Cornille, F., Lenoir, C. and Roques, BP. "High-Throughput Fluorogenic Assay for Determination of Botulinum Type B Neurotoxin Protease Activity", (2001), *Anal. Biochem.* **291**:253-261.

Reconstitution

A small amount of peptide has been lyophilized in each vial. During lyophilization and transportation, this material may be distributed throughout the vial. Since it is common practice to reconstitute peptide in a small volume of solvent, visually locate the powder and, if necessary, shake it to the bottom of the vial prior to adding the solvent. It is recommended that initial stock solutions be made in 20% dimethylformamide (DMF) to ensure total recovery of the product from the vial. Cover the vial with foil to protect from light.

Concentration

Concentration is determined using the peptide content.

Analysis

The peptide is >95% pure as determined by reverse phase HPLC. The expected molecular weight was obtained by mass spectrometry.

Assay Conditions and Parameters for Utilizing VAMPtide® (PL 150, Pya/Nop) Peptide:

VAMPtide® (PL 150, Pya/Nop), Product #542

Prepare a 1 mM stock solution of this peptide in 20% (v/v) dimethylformamide (DMF) as follows: Add 200 µl of 20% DMF to a vial containing 200 nmoles of peptide. Mix gently until the solution is clear. Cover the vial with foil to protect from light, and store frozen at -20°C.

Assays for protease activity of both BoNT/B Light Chain and BoNT/B holotoxin are performed using 20 mM HEPES, pH 7.4 containing 2 µM ZnSO₄ and 1.5 mM Tris (2-carboxyethyl) phosphine (TCEP) as the hydrolysis buffer. The HEPES buffers are prepared by titrating the free acid form of HEPES with the potassium salt form of HEPES. The 1 mM stock solution of VAMPtide® (PL 150, Pya/Nop) is diluted in the hydrolysis buffer so that the final concentration is typically between 5 µM and 10 µM.

The hydrolysis assays are run at 37°C. Excitation wavelength is 343 nm and emission is 397 nm.

(continued)

Botulinum Neurotoxin Type B Light Chain, Recombinant, Product #620A

For the reconstitution of BoNT/B Light Chain stock solutions, use 20 mM HEPES, pH 7.4 containing 0.05% TWEEN 20 or 1 mg/ml BSA. The addition of TWEEN 20 or BSA is beneficial to the recovery, stability and storage of reconstituted BoNT/B Light Chain at -20°C. For the hydrolysis reaction of VAMPtide® (PL 150, Pya/Nop) with BoNT/B Light Chain, use the hydrolysis buffer 20 mM HEPES, pH 7.4, containing 2 µM ZnSO₄ and 1.5 mM TCEP. Under these conditions, 10 µM VAMPtide® (PL 150, Pya/Nop) is totally digested by 1.25 nM BoNT/B Light Chain in less than one hour.

Botulinum Neurotoxin Type B (BoNT/B), Product #136

BoNT/B is reconstituted in 20 mM HEPES, pH 7.4, 0.2% TWEEN 20. The addition of TWEEN 20 to the reconstitution buffer is beneficial to the recovery of BoNT/B from the vial. The reaction buffer for hydrolysis of VAMPtide® using BoNT/B is 20 mM HEPES, pH 7.4, containing 2 µM ZnSO₄ and 1.5 mM TCEP. BoNT/B does not require an extra incubation period for reduction and can be used immediately after reconstitution in the reaction buffer.

For further information regarding VAMPtide®, Product #542, digestion with the BoNT/B Light Chain and holotoxin, click on the Posters tab on our website, www.listlabs.com and view the poster entitled, "Evaluation of Three VAMPtide® Substrates for Botulinum Neurotoxin Type B."

Related Products

Product #540: VAMPtide® with an o-Abz/Dnp FRET pair

Product #541: VAMPtide® with a FITC/DABCYL FRET pair

Product #549: Calibration Fluorophore for VAMPtide® 540

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 for use 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 USE IN HUMANS.

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