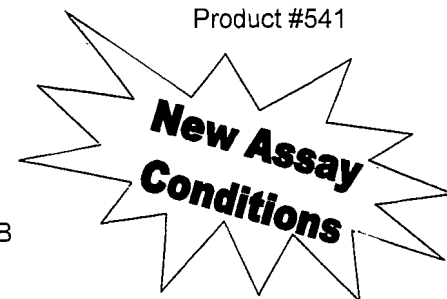


CERTIFICATE OF ANALYSIS
VAMPtide® (FITC/DABCYL)
Peptide Substrate for Botulinum Neurotoxin Type B
Lot #5411A1

Product #541



Contents:

Each vial of VAMPtide® (FITC/DABCYL), a botulinum neurotoxin type B (BoNT/B) substrate, contains 200 nmoles of lyophilized peptide. This peptide is intramolecularly quenched by fluorescence resonance energy transfer (FRET). The N-terminally-linked fluorophore is fluorescein-thiocarbamoyl (FITC), and the acceptor chromophore is DABCYL. This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C.

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 DMSO to ensure total recovery of the product from the vial. Cover the vial with foil to protect from light.

Concentration:

Peptide content is obtained from nitrogen determination.

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® (FITC/DABCYL) FRET Peptide:

VAMPtide® (FITC/DABCYL), Product #541

Prepare a 2.5 mM stock solution of this peptide in DMSO as follows: Add 80 µl of DMSO to a vial containing 200 nmoles of peptide. Cover the vial with foil to protect from light, and store frozen at -20°C.

The FRET assays are performed using HEPES buffers prepared by titrating the free acid form of HEPES with the potassium salt form of HEPES. For assays with BoNT/B holotoxin, the VAMPtide® stock solution is diluted to a maximum of 50 µM in 20 mM HEPES, pH 6.3. For assays with BoNT/B Light Chain, the VAMPtide® stock solution is diluted to a maximum of 50 µM in the hydrolysis buffer, 50 mM HEPES, pH 6.3, 0.05% TWEEN 20. The lower pH is necessary to fully dissolve the peptide substrate and with BoNT/B Light Chain, optimum cleavage is observed at this pH value. The final concentration of VAMPtide® to be used is 5 µM/well. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the total volume.

FRET assays using BoNT/B holotoxin are run at 37°C. FRET assays with BoNT/B Light Chain are run at 25°C. Non-enzymatic cleavage of Prod #541 is sometimes observed at temperatures above 25°C using the BoNT/B Light Chain hydrolysis buffer. Excitation wavelength is 490 nm and emission is 523 nm with a cutoff filter at 495 nm.

When measuring kinetic parameters such as K_m and V_{max} for this FRET substrate, the data must be corrected for a phenomenon known as the "inner filter effect". This effect, as well as a method to determine an appropriate correction factor, is explained in the paper by Liu *et al* (1999) in *Analytical Biochemistry*, **267**, 331-335.

(continued)

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 0.05 mM ZnSO₄, 5 mM DTT. BoNT/B does not require an extra incubation period for reduction and can be used immediately after reconstitution in the reaction buffer.

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

For the reconstitution of BoNT/B Light Chain and for the hydrolysis reaction of VAMPtide® with BoNT/B Light Chain, use the hydrolysis buffer 50 mM HEPES, pH 6.3, containing 0.05% TWEEN 20. BoNT/B Light Chain does not require reduction. Concentrations of BoNT/B Light Chain between 5 nM and 10 nM can be used depending on the instrumentation and experiment. The addition of 0.05% TWEEN 20 or 1 mg/ml BSA is beneficial to the stability and storage of reconstituted BoNT/B Light Chain at -20°C.

Related Products:

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

Product #549: Calibration Fluorophore for VAMPtide® 540

For further information regarding this FRET peptide and related products, click on the Posters tab on our website. www.listlabs.com.

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. 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.

Production: VFC Date: 12/16/11 Management: SS Date: 12/16/11 QA/QC: CL Date: 12/16/11