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Product #540

CERTIFICATE OF ANALYSIS
VAMPtide® (o-Abz/Dnp)
Peptide Substrate for Botulinum Neurotoxin Type B
Lot #5404A1

Contents:

Each vial of VAMPtide® (o-Abz/Dnp), 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 o-aminobenzoic acid (o-Abz) and the acceptor chromophore is a 2,4-dinitrophenyl group (Dnp). This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light.

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:

Concentration is determined from the absorbance at 363 nm using the molar absorption coefficient of 15,900 $M^{-1}cm^{-1}$ for Lys (Dnp).

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® (o-Abz/Dnp) FRET Peptide:

VAMPtide® (o-Abz/Dnp), Product #540

Prepare a 5 mM stock solution of this peptide in DMSO as follows: Add 40 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, the VAMPtide® stock solution is diluted using 20 mM HEPES, pH 6.3, prior to use. The lower pH is necessary to fully dissolve the peptide substrate. For assays with BoNT/B Light Chain, the stock solution should be diluted in the hydrolysis buffer described in the section below. When using a 96-well plate and final volume of 250 µL/well, a 250 µM stock solution is convenient to use. The final concentration of VAMPtide® to be used is typically between 5 µM and 10 µM/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the total volume. For VAMPtide® (o-Abz/Dnp), Prod #540, any concentration of $ZnCl_2$ in the BoNT/B Light Chain hydrolysis buffer inhibits cleavage.

These FRET assays are run at 37°C. Excitation wavelength is 320 nm and emission is 418 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

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an appropriate correction factor, is explained in the paper by Liu *et.al* (1999) in *Analytical Biochemistry,* **267,** 331-335. The correction method uses an unquenched fluorophore for comparison. Since the fluorescence efficiency for the free o-Abz is higher than that for o-Abz when it is bound to an amino acid, the use of product #549, Calibration Fluorophore for VAMPtide® 540, in the place of the free o-Abz, is suggested. This peptide contains the o-Abz bound to a single amino acid.

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 $ZnSO_4$, 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 #541: VAMPtide® containing a FITC/DABCYL 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.

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