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Product #540

CERTIFICATE OF ANALYSIS
VAMPtide® (o-Abz/Dnp)
Peptide Substrate for Botulinum Neurotoxin Type B
Lot #5403A1

Contents:

Each vial of VAMPtide®, the botulinum toxin type B substrate, contains 200 nmoles of lyophilized peptide. The solubility is ~ 200 µM in water. Higher concentrations may be achieved in DMSO. It is recommended that initial stock solutions be made in DMSO to insure total recovery of lyophilized peptide. This peptide is intramolecularly quenched by fluorescence resonance energy transfer (FRET). The fluorophore is o-aminobenzoic acid (o-Abz), and the acceptor chromophore is 2,4-dinitrophenyl (Dnp).

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, we suggest visually locating the peptide and, if necessary, shaking it to the bottom of the vial prior to adding the solvent.

Concentration:

Concentration is determined from the absorbance at 363 nm using the molar absorption coefficient of 15900 $M^{-1}cm^{-1}$ for Lys(Dnp).

Purity:

The peptide is >90% pure as determined by reverse phase HPLC. The expected molecular weight is obtained by mass spectrometry.

Assay Condition and Parameters for Utilizing VAMPtide® FRET Peptide :

Botulinum Neurotoxin Type B, Holotoxin (BTB), product #136A

BTB is reconstituted in 20 mM HEPES, pH 7.4, 0.2% TWEEN-20. The reaction buffer for hydrolysis of VAMPtide® by BTB is 20 mM HEPES, containing 0.05 mM $ZnSO_4$, 5 mM DTT. Using this reaction buffer BTB does not require an extra incubation period for reduction. Concentrations of BTB between 5 nM and 10 nM can be used depending on the instrumentation and experiment. Our data suggest that addition of TWEEN-20 to the reconstitution buffer is beneficial to the recovery of BTB from the vial. All HEPES buffers are obtained by titrating the free acid form of HEPES with the potassium salt form of HEPES.

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

For the reconstitution of Light Chain B (LcB) and for the hydrolysis reaction of VAMPtide® with LcB, use hydrolysis buffer 50 mM HEPES, pH 6.3, containing 0.05% TWEEN-20. The pH 6.3 HEPES buffer was obtained by titrating the free acid form of HEPES with the potassium salt form of HEPES. LcB does not require reduction. Concentrations of LcB between 5 nM and 10 nM can be used depending on the instrumentation and experiment. Our data suggest that addition of TWEEN-20 or BSA is beneficial to the stability and storage of LcB at -20°C.

(continued)

VAMPtide® (o-Abz/Dnp), Product #540

Prepare a 5 mM stock solution of this peptide in DMSO as follows: Add 40 µl of DMSO, Pierce cat. #20684, to a vial containing 200 nmoles of peptide. The resulting stock solution is 5 mM. Cover the vial with foil to protect from light and store frozen at -20°C.

For assays with LcB, the stock solution is diluted in 50 mM HEPES, pH 6.3, 0.05% TWEEN-20. For assays with BTB, the stock solution is diluted in 20 mM HEPES, pH 6.3. The lowered pH is necessary for solution of the peptide substrate. 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® 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 that 2% of the volume. The FRET assays are run at 37°C. Excitation wavelength is 321 nm and emission is 418 nm.

When measuring kinetic parameters such as K_m and V_{max} for this 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) *Analytical Biochemistry,* **267,** 331-335.

Packing/Storage:

This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light. After reconstitution, aliquot and store at -20°C.

Handling:

This product is not known to be hazardous. Good laboratory technique should be employed in handling of this product. This requires observing the following practices:

1. **Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.**

2. **Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.**

3. **Avoid accidental autoinoculation by exercising care when handling in conjunction with any injection device.**

4. **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.

Approved: EE Date: 08/11/09 Approved: NS Date: 8/11/09