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**CERTIFICATE OF ANALYSIS**  
**SYNTAXtide® (o-Abz/Dnp)**  
**Peptide Substrate for Botulinum Neurotoxin Type C**  
**Lot #5602A1**

**Contents:**

Each vial of SYNTAXtide® (o-Abz/Dnp), a botulinum neurotoxin type C (BoNT/C) 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<sup>-1</sup>cm<sup>-1</sup> 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 SYNTAXtide® (o-Abz/Dnp) FRET Peptide:**

**SYNTAXtide® (o-Abz/Dnp), Product #560**

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/C Light Chain, the SYNTAXtide® stock solution is diluted in the hydrolysis buffer, 20 mM HEPES, pH 6.8, containing 0.05% TWEEN 20. When using a 96-well plate and a final volume of 250 µL/well, a 250 µM stock solution is convenient to use. The final concentration of SYNTAXtide® to be used is typically 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 SYNTAXtide® (o-Abz/Dnp), Prod #560, any concentration of ZnCl<sub>2</sub> in the hydrolysis buffer inhibits cleavage. These FRET assays are run at room temperature. Excitation wavelength is 320 nm and emission is 420 nm.

**Botulinum Neurotoxin Type C Light Chain, Recombinant, Product #625A**

For the reconstitution of BoNT/C Light Chain use 20 mM HEPES, pH 7.4, 0.05% TWEEN 20. For the hydrolysis reaction of SYNTAXtide® with BoNT/C Light Chain, use the hydrolysis buffer 20 mM HEPES, pH 6.8, containing 0.05% TWEEN 20. BoNT/C Light Chain does not require reduction. Concentrations of BoNT/C Light Chain between 5 nM and 10 nM can be used depending on the instrumentation and experiment. The hydrolysis reaction of SYNTAXtide® with BoNT/C Light Chain can be measured hourly followed by an overnight read at room temperature. The addition of 0.05% TWEEN 20 or 1 mg/mL BSA is beneficial to the stability and storage of reconstituted BoNT/C Light Chain at -20°C.

(continued)

For further information regarding this FRET peptide and related products, click on the Posters tab on our website, [www.listlabs.com](http://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 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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