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Product #560

CERTIFICATE OF ANALYSIS
SYNTAXtide™(o-Abz/Dnp)
Peptide Substrate for Botulinum Neurotoxin Type C
Lot #5601A1

Contents:

Each vial of the botulinum toxin type C substrate, SYNTAXtide™(o-Abz/Dnp) contains 200 nmoles of lyophilized peptide. The solubility is ~1.4 mM in water. Higher concentrations may be achieved in DMSO. 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).

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 powder 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 15,900 M⁻¹cm⁻¹ for Lys(Dnp).

Purity:

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

Assay Conditions and Parameters for Utilizing SYNTAXtide™FRET Peptide:

Botulinum Neurotoxin Type C Light Chain, Recombinant (LcC), Product #625A

For the reconstitution of Light Chain C (LcC) use 20 mM HEPES, pH 7.4, 0.05% TWEEN-20. For the hydrolysis reaction of SYNTAXtide™with LcC, use the hydrolysis buffer 20 mM HEPES, pH 6.8, containing 0.05% TWEEN 20 (or 1 mg/ml BSA). LcC does not require reduction. Concentrations of LcC between 2 nM and 10 nM can be used depending on the instrumentation and experiment. Our data suggests that addition of TWEEN 20 or BSA is beneficial to the stability and storage of LcC at -20°C.

SYNTAXtide™, Product #560

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.

(continued)

For assays with LcC, the stock solution can be diluted in the hydrolysis buffer, described in the section above. When using a 96-well plate and a final volume of 250 μ l/well, a 250 μ M stock solution is convenient to use. The final concentrations of SYNTAXtide™ to be used is typically 5 μ M/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the total volume.

These FRET assays are run at 37°C. Excitation wavelength is 320 nm and emission is 420 nm. There is a linear dependence of fluorescence intensity on concentration of totally cleaved substrate up to 10 μ M SYNTAXtide™(o-Abz/Dnp).

When measuring kinetic parameters such as the 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.

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 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.

Production: _____ Date: _____ Management: _____ Date: _____ QC: _____ Date: _____