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Product #521

CERTIFICATE OF ANALYSIS
SNAPtide®(FITC/DABCYL)
Peptide Substrate for Botulinum Neurotoxin Type A
Lot #5213A2

Contents:

Each vial of the botulinum toxin type A substrate, SNAPtide® (FITC/DABCYL), (U.S. Patent #6,504,006) contains 200 nmoles of lyophilized peptide. In order to maximize recovery from the vial, and for stability in storage, stock solutions (2.5-5 mM) should be prepared in DMSO. 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. Note: SNAPtide® containing an o-Abz/Dnp FRET pair is also available.

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:

Peptide content is determined from amino acid analysis.

Purity:

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

Assay Conditions and Parameters for Utilizing SNAPtide® FRET Peptide

Botulinum Neurotoxin Type A (BTA), Product #130A
We suggest reconstituting this protein with the reduction buffer, 20 mM HEPES, pH 8.0, containing 5 mM DTT, 0.3 mM $ZnCl_2$, and 1.0 mg/ml BSA. In order to activate BTA it must be reduced by incubation for 30 minutes at 37°C immediately following reconstitution in this buffer. Use reduced toxin as soon as possible. Concentrations of BTA between 2 nM and 10 nM can be used depending on the instrumentation and experiment. The BSA in the reduction buffer is essential for recovery of BTA, product #130A (10 µg), from the vial. It is possible to substitute 0.2% TWEEN-20 for the BSA.

The reaction buffer for hydrolysis of SNAPtide® using BTA is 20 mM HEPES, containing 0.3 mM $ZnCl_2$, 1.25 mM DTT and 0.1% TWEEN-20, pH 8.0. All HEPES buffers are obtained by titrating the free acid form of HEPES with the potassium salt form of HEPES.

Botulinum Neurotoxin Type A Light Chain, Recombinant (LcA), Product #610A
For the reconstitution of Light Chain A (LcA) and for the hydrolysis reaction of SNAPtide® with LcA, use the hydrolysis buffer 20 mM HEPES, pH 8.2, containing 0.5 mg/ml BSA (or 0.1% TWEEN-20). LcA does not require reduction. Concentrations of LcA 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 LcA at -20°C.

(continued)

SNAPtide® (FITC/DABCYL) product #521

Prepare a 2.5 mM stock solution of this peptide in DMSO as follows: Add 80 µl of DMSO, Pierce catalog #20684, to a vial containing 200 nmoles of peptide. The resulting stock solution is 2.5 mM. Cover the vial with foil to protect from light, and store frozen at -20°C.

For assays with BTA, the stock solution is diluted using 20 mM HEPES, pH 8.0, prior to use. For assays with LcA, the stock solution can be diluted in 20 mM HEPES, pH 8.2. 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 concentrations of SNAPtide® to be used is typically between 5 µM and 8 µM/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the volume.

These FRET assays are run at 37°C. Excitation wavelength is 490 nm and emission is 523 nm with a cutoff filter at 495 nm. There is a linear dependence of fluorescence intensity on concentration of totally cleaved substrate up to ~12 µM SNAPtide® (FITC/DABCYL).

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) *Analytical Biochemistry,* **267,** 331-335. Since the fluorescence efficiency for the free FITC is higher than for FITC when it is bound to the peptide, the use of product #528, SNAPtide® Unquenched Calibration Peptide for #521, in the place of the free FITC, is suggested. This peptide contains the FITC bound to the N-terminal cleaved fragment of SNAPtide®.

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.

Approved: FE Date: 08/10/09 Approved: NS Date: 8/11/09