

CERTIFICATE OF ANALYSIS
SNAPtide® fIP6 (DABCYL/5-IAF), Prod #523
Peptide Substrate for Botulinum Neurotoxin Type A
Lot #5232A1

Contents

Each vial of SNAPtide® fIP6 (DABCYL/5-IAF), a botulinum neurotoxin type A (BoNT/A) substrate, contains 200 nmoles of lyophilized peptide. This peptide is intramolecularly quenched by fluorescence resonance energy transfer (FRET). This peptide is labeled using 5-iodoacetamido-fluorescein to obtain an S-fluoresceinyl cysteine fluorophore on the C-terminus. The acceptor chromophore is DABCYL. 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:

Peptide content is obtained from nitrogen determination.

Analysis:

The peptide is ≥ 95% pure as analyzed by reversed phase HPLC. The expected molecular weight was verified by mass spectrometry.

Assay Conditions and Parameters for Utilizing SNAPtide® fIP6 (DABCYL/5-IAF) FRET Peptide:

SNAPtide® fIP6 (DABCYL/5-IAF), Product #523

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/A holotoxin, the SNAPtide® fIP6 (DABCYL/5-IAF) stock solution is diluted with the reaction buffer, 20 mM HEPES, pH 7.4, 0.15 mM ZnCl₂, 1.25 mM DTT and 0.1% TWEEN 20. This dilution should not exceed 50µM due to limited solubility of the substrate at this optimum pH for the reaction. The final concentration of SNAPtide® fIP6 (DABCYL/5-IAF) to be used is 5-8 µM/well. Since DMSO inhibits cleavage, the final concentration must be less than 2% of the total volume.

The FRET assay is 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 ~8 µM SNAPtide® fIP6 (DABCYL/5-IAF).

(continued)

Botulinum Neurotoxin Type A (BoNT/A), Product #130

It is recommended to reconstitute this protein with the reduction buffer, 20 mM HEPES, pH 8.0, containing 5 mM DTT, 0.3 mM ZnCl₂ and 0.1% TWEEN 20. In order to activate BoNT/A 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 BoNT/A between 2 nM and 10 nM can be used depending on the instrumentation and experiment. The TWEEN 20 in the reduction buffer is essential for recovery of the BoNT/A from the vial. It is possible to substitute 1 mg/ml BSA for the TWEEN 20.

The reaction buffer for hydrolysis of SNAPtide® fIP6 (DABCYL/5-IAF) using BoNT/A is 20 mM HEPES, pH 7.4, containing 0.15 mM ZnCl₂, 1.25 mM DTT and 0.1% TWEEN 20.

Related Products:

Product #520: SNAPtide® containing an o-Abz/Dnp FRET pair (U.S. Patent #6,504,006).

Product #521: SNAPtide® containing a FITC/DABCYL FRET pair (U.S. Patent #6,504,006).

Product #525: Control Peptide for SNAPtide® 520 for C. botulinum Type A Neurotoxin. A control peptide containing two amino acid substitutions, which is not a substrate for botulinum neurotoxin type A, however, is an ideal control peptide since it contains all of the sites for non-specific cleavage found in SNAPtide® #520.

Product #526: Control Peptide for SNAPtide® 521 for C. botulinum Type A Neurotoxin. A control peptide containing two amino acid substitutions, which is not a substrate for botulinum neurotoxin type A, however, is an ideal control peptide since it contains all of the sites for non-specific cleavage found in SNAPtide® #521.

Product #528: UNQUENCHED CALIBRATION PEPTIDE FOR SNAPtide® 521. A calibration peptide which is the cleavage product of SNAPtide® containing only the FITC at the N-terminal; it can be used to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

Product #529: UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 520. A calibration peptide which is the cleavage product of SNAPtide® containing only the o-Abz at the N-terminal; it can be used to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

For further information regarding this FRET peptide and related products, visit the website at www.listlabs.com. Posters available upon request.

Handling:

This product is not known to be hazardous. Good laboratory technique should be employed in the safe handling of the product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended 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 the product.

FOR RESEARCH PURPOSES ONLY. NOT FOR USE IN HUMANS

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