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CERTIFICATE OF ANALYSIS
SNAPTide® fIP6 (DABCYL/5-IAF)
Peptide Substrate for Botulinum Neurotoxin Type A
Lot #5231A1

Product #523



**New Assay
Conditions**

Contents:

Each vial of SNAPTide® fIP6 (DABCYL/5-IAF) (U.S. Patent Pending #61/252,675), 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-terminal. 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 peptides 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 determined by reverse phase HPLC. The expected molecular weight was obtained 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 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. For assays with BoNT/A Light Chain, the SNAPTide® fIP6 stock solution is diluted with 50 mM HEPES, pH 7.4, containing 0.05% TWEEN 20 and 0.5 mg/ml BSA. These dilutions should not exceed 50 µM 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, final concentrations must be less than 2% of the total 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 ~8 µM SNAPTide® fIP6 (DABCYL/5-IAF).

SNAPTide® fIP6 (DABCYL/5-IAF) is suitable for use in kinetic measurements. In studies using BoNT/A Light Chain, it displays saturable Michaelis Menten kinetics; K_m is approximately 3.8 µM as opposed to >200 µM for Prod. #521.

(continued)

Botulinum Neurotoxin Type A (BoNT/A), Product #130/#9130

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 using BoNT/A is 20 mM HEPES, pH 7.4, containing 0.15 mM ZnCl₂, 1.25 mM DTT and 0.1% TWEEN 20.

Botulinum Neurotoxin Type A Light Chain, Recombinant, Product #610A

For the reconstitution of BoNT/A Light Chain and for the hydrolysis reaction of SNAPtide® fIP6 (DABCYL/5-IAF) with BoNT/A Light Chain, use the hydrolysis buffer 50 mM HEPES, pH 7.4, containing 0.05% TWEEN 20 and 0.5 mg/ml BSA. BoNT/A Light Chain does not require reduction. Concentrations of BoNT/A Light Chain between 2 nM and 10 nM can be used depending on the instrumentation and experiment. The addition of 0.05% TWEEN 20 or 1 mg/ml BSA is beneficial to the stability and storage of reconstituted BoNT/A Light Chain at -20°C.

Recent studies analyzing the effect of the osmolyte, trimethylamine N-oxide (TMAO), on the rate of cleavage of SNAPtide® fIP6, Prod #523 with BoNT/A Light Chain indicate that the rate decreases dramatically when TMAO is added to the FRET substrate prior to the BoNT/A Light Chain. This is in direct contrast to SNAPtide®, Prod #521, where a significant increase in the rate of cleavage is observed in the presence of 2.3 M TMAO.

Related Products:

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

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

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, click on the Posters tab on our website. 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 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 this product.

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