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Product #521
Lot No #5214A3

CERTIFICATE OF ANALYSIS
SNAPtide® (FITC/DABCYL)
Peptide Substrate for Botulinum Neurotoxin Type A
Lot #5214A3

Contents

Each vial of SNAPtide® (FITC/DABCYL) (U.S. Patent #6,504,006), 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). The N-terminally-linked fluorophore is fluorescein-thiocarbamoyl (FITC) and 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 80% 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® (FITC/DABCYL) FRET Peptide

SNAPtide® (FITC/DABCYL), Product #521

Prepare a 2.5 mM stock solution of this peptide in 80% DMSO as follows: Add 80 µl of 80% 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® stock solution is diluted with the reaction buffer, 20 mM HEPES, pH 7.4, 0.15 mM of $ZnCl_2$, 1.25 mM DTT, and 0.1% TWEEN 20. For assays with BoNT/A Light Chain, the stock solution should be diluted in 50 mM HEPES, pH 7.4, 0.05% TWEEN 20. These dilutions should not exceed 50 µM due to limited solubility of the substrate at this optimum pH (7.4) for the reaction. The final concentration 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 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 ~12 µM SNAPtide® (FITC/DABCYL).

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

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 $ZnCl_2$ and 1 mg/ml BSA. 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 BSA in the reduction buffer is essential for recovery of BoNT/A from the vial. It is possible to substitute 0.1% TWEEN 20 for the BSA.

The reaction buffer for hydrolysis of SNAPtide® using BoNT/A is 20 mM HEPES, pH 7.4, containing 0.15 mM $ZnCl_2$, 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® with BoNT/A Light Chain, use the hydrolysis buffer 50 mM HEPES, pH 7.4, containing 0.05% TWEEN 20. 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®, Prod #521, with BoNT/A Light Chain indicate that the rate increases approximately 3-fold when the TMAO is added to the FRET substrate prior to the BoNT/A Light Chain. The maximum effect is observed in the presence of 2.3 M TMAO.

The dissociation constant, K_m, is greater than 200 µM when measured in the absence of TMAO. In the presence of 2.3 M TMAO, the K_m is significantly decreased to ~4 µM.

The detection limit, calculated as 2.998 (Student's t-Distribution with 99% confidence and 7 degrees of freedom) times the standard deviation of 8 replicates, is 0.4 ng/ml after 4.5 hours digestion of 8 µM SNAPtide® at 37°C and 0.3 ng/ml after continuing digestion overnight at room temperature. In the presence of 2.3 M TMAO, under the same conditions, 0.16 ng/ml is detected in 4.5 hours and after continued digestion overnight at room temperature.

Related Products

Product #526: CONTROL PEPTIDE for SNAPtide® 521. The control peptide for SNAPtide® 521 contains two substitutions and is not a substrate for BoNT/A, however, since it contains all of the sites for non-specific cleavage found in SNAPtide® 521, it is an ideal control peptide.

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).

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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 can be used to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

Product #523: SNAPtide® flP6 containing a DABCYL/5-IAF FRET pair (U.S. Patent Pending #61/252,675).

For further information regarding this FRET peptide and related products, visit our website at 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 or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

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Quality Assurance: _____ Date: 08 JUL 2021