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Certificate of Testing

SNAPtide® (o-Abz/DNP) Peptide Substrate for Botulinum Neurotoxin Type A

Prod. No:	520	**Lot Number:**	5209A4
Date of Manufacture	06 August 2021	**FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.**	

Contents
Each vial of SNAPtide® (o-Abz/Dnp) (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 o-aminobenzoic acid (o-Abz) and the acceptor chromophore is a 2,4-dinitrophenyl group (Dnp). This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light.

Concentration
Concentration is determined from the absorbance at 363 nm using the molar absorption coefficient of 15,900 $M^{-1}cm^{-1}$ for Lys(Dnp).

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® (o-Abz/Dnp) FRET Peptide

SNAPtide® (o-Abz/Dnp), Product #520
Prepare a 5 mM stock solution of this peptide in DMSO as follows: Add 40 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 botulinum neurotoxin Type A (BoNT/A) holotoxin, the SNAPtide® stock solution is diluted using 20 mM HEPES, pH 8.0, prior to use. For assays with BoNT/A Light Chain, the stock solution should be diluted in the hydrolysis buffer, described in the section below. 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 concentration of SNAPtide® to be used is typically between 5 µM and 10 µM/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the total volume. For SNAPtide® (o-Abz/Dnp), Product #520, any concentration of $ZnCl_2$ in the BoNT/A Light Chain hydrolysis buffer inhibits cleavage.

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 30 µM SNAPtide® (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. The correction method uses an unquenched fluorophore for comparison. Since the fluorescence efficiency for the free o-Abz is higher than that for o-Abz when it is bound to the peptide, the use of product #529, Unquenched Calibration Peptide for SNAPtide® 520, in the place of the free o-Abz, is suggested. This peptide contains the o-Abz bound to the N-terminal cleaved fragment of SNAPtide®.

Botulinum Neurotoxin Type A (BoNT/A) Holotoxin, 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 $ZnSO_4$ and 0.1% TWEEN 20. In order to activate BoNT/A holotoxin 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 holotoxin 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 holotoxin from the vial. It is possible to substitute 1 mg/ml BSA for the TWEEN 20.

The reaction buffer for the hydrolysis of SNAPtide® using BoNT/A holotoxin is 20 mM HEPES, pH 8.0, containing 0.75 mM $ZnSO_4$, 1.25 mM DTT and 0.1% TWEEN 20. After 3.5 hours of digestion at 37°C, 3 ng/ml BoNT/A holotoxin can be observed. After continuing the digestion overnight at room temperature, the lowest concentration analyzed, 750 pg/ml, is easily observed.

Botulinum Neurotoxin Type A Light Chain, GST Fusion, Recombinant, Product #611A

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.

The limit of detection (LOD) defined as the concentration of BoNT/A Light Chain required to give a signal equal to the blank plus three times the standard deviation of the blank, is 0.2 ng/ml BoNT/A Light Chain after 5 hours digestion of 10 µM SNAPtide® at 37°C, and 0.08 ng/ml after continuing digestion overnight at room temperature. Using 20 µM SNAPtide® under the same conditions, 0.1 ng/ml is detected in 5 hours and 0.05 ng/ml after continued digestion overnight at room temperature.

Note: Recent studies analyzing the effect of the osmolyte, trimethylamine N-oxide (TMAO), on the rate of cleavage of SNAPtide®, Prod #520 with BoNT/A Light Chain indicate that the rate decreases as the concentration of the TMAO increases. The opposite effect is observed with SNAPtide®, Prod #521.

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.

Quality Control Manager: _____ Date: 08/26/2021

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