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Certificate of Testing

SNAP Etide® (o-Abz/Dnp) Peptide Substrate for Botulinum Neurotoxin Type E

Prod. No:	550	**Lot Number:**	5504A1
Date of Manufacture	16 April 2024	**FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.**	

Contents

Each vial of SNAP Etide® (o-Abz/Dnp), a botulinum neurotoxin type E (BoNT/E) substrate, contains 100 nmoles of lyophilized peptide. This peptide is intramolecularly quenched by fluorescence resonance energy transfer (FRET). The fluorophore is o-aminobenzoic acid (o-Abz) and the acceptor chromophore is 2,4-dinitrophenol (Dnp). 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

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 analyzed by reversed phase HPLC. The expected molecular weight was verified by mass spectrometry.

Assay Conditions and Parameters for utilizing SNAP Etide® (o-Abz/Dnp) FRET Peptide

SNAP Etide® (o-Abz/Dnp), Product #550

Prepare a 5 mM stock solution of this peptide in DMSO as follows: Add 20 µL of DMSO to a vial containing 100 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/E holotoxin, the SNAP Etide® stock solution is diluted with the reaction buffer, 50 mM HEPES, pH 8.0, containing 0.6 mM $ZnCl_2$, 1.25 mM DTT and 0.1% TWEEN 20. For assays with BoNT/E Light Chain, the SNAP Etide® stock solution is diluted in the hydrolysis buffer, 50 mM HEPES, pH 7.8, containing 0.1% TWEEN 20. When using a 96-well plate and final volume of 250 µL/well, a 125 µM stock solution is convenient to use. The final concentration of SNAP Etide® to be used is typically 5 µL/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations must be less than 2% of the total volume. For SNAP Etide® (o-Abz/Dnp), Product #550, any concentration of $ZnCl_2$ in the BoNT/E Light Chain hydrolysis buffer inhibits cleavage.

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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 ~25 µM SNAP Etide® (o-Abz/Dnp).

When measuring kinetic parameters such as 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 an amino acid, the use of Product #559, Unquenched Calibration Peptide for SNAP Etide®, in the place of the free o-Abz, is suggested. This peptide is identical to the N-terminal cleavage product containing the fluorophore, o-aminobenzoic acid (o-Abz), which results from botulinum neurotoxin type E hydrolysis of the SNAP Etide® (o-Abz/Dnp).

Botulinum Neurotoxin Type E (BoNT/E), Product #141A

It is recommended to reconstitute this protein with the reduction buffer, 50 mM HEPES, pH 8.0, containing 5 mM DTT, 0.3 mM $ZnCl_2$ and 0.1% TWEEN 20. In order to activate BoNT/E, 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/E between 5 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/E from the vial. It is possible to substitute 1 mg/ml BSA for the TWEEN 20. The reaction buffer for hydrolysis of SNAP Etide® using BoNT/E is 50 mM HEPES, pH 8.0, containing 0.6 mM $ZnCl_2$, 1.25 mM DTT and 0.1% TWEEN 20.

Botulinum Neurotoxin Type E (BoNT/E) Light Chain, Recombinant

For the reconstitution of BoNT/E Light Chain and for the hydrolysis reaction of SNAP Etide® with BoNT/E Light Chain, use the hydrolysis buffer 50 mM HEPES, pH 7.8, containing 0.1% TWEEN 20. BoNT/E Light Chain does not require reduction. Concentrations of BoNT/E Light Chain between 1 nM and 5 nM can be used depending on the instrumentation and experiment. The addition of 0.1% TWEEN 20 or 1 mg/ml BSA is beneficial to the stability and storage of reconstituted BoNT/E Light Chain at −20°C.

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.

Quality Control: _____ Date: 05/01/2024

Production: Isabel Christin Date: 05/01/2024