

CERTIFICATE OF ANALYSIS  
CONTROL PEPTIDE for SNAPtide® 520  
Lot #5252A1



**Contents:**

Each vial of the CONTROL PEPTIDE for SNAPtide® 520 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.

This CONTROL PEPTIDE is identical to SNAPtide® Peptide Substrate (o-Abz/Dnp) for *Clostridium botulinum* Type A Neurotoxin (BoNT/A), Prod #520, except for two substitutions which eliminate hydrolysis by BoNT/A. This CONTROL PEPTIDE is not a substrate for BoNT/A, however, since it contains all of the sites for non-specific cleavage found in SNAPtide® (o-Abz/Dnp) Prod #520, it is an ideal control peptide. The cleavage of Product #525 by BoNT/A is severely compromised. All potential non-specific cleavage sites remain available in the Product #525 sequence. This CONTROL PEPTIDE can be used to screen background cleavage of SNAPtide® (o-Abz/Dnp) Prod #520, that can occur in complex matrices limiting the detection of low amounts of BoNT/A.

**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 lyophilized peptide. 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<sup>-1</sup>cm<sup>-1</sup> for Lys(Dnp).

**Purity:**

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 CONTROL PEPTIDE for SNAPtide® 520:**

The CONTROL PEPTIDE for SNAPtide® 520, Prod #525, is used in conjunction with SNAPtide® Peptide Substrate (o-Abz/Dnp) for BoNT/A, Prod #520, and therefore the CONTROL PEPTIDE (Prod #525) should be prepared exactly the same as the peptide substrate (Prod #520). The assay conditions outlined below are identical to the conditions given for Prod #520.

**CONTROL PEPTIDE for SNAPtide® 520, Product #525**

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 BoNT/A holotoxin and BoNT/A Light Chain, the stock solution can be diluted in the hydrolysis buffer, described in the sections 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 concentrations of CONTROL PEPTIDE for SNAPtide® 520 should be the same as that of Prod #520 used in the experiment.

The FRET assays are run at 37°C. Excitation wavelength is 320 nm and emission is 420 nm.

Evaluation of the CONTROL PEPTIDE for SNAPtide® 520 and comparison studies with the SNAPtide® (o-Abz/Dnp) Prod #520 substrate are discussed in the poster entitled:

EVALUATION OF A SMALL PEPTIDE INHIBITOR AND A CONTROL PEPTIDE FOR BOTULINUM NEUROTOXIN, TYPE A Nancy Shine, Kayana Suryadi and Todd Christian presented at the 47<sup>th</sup> Annual Interagency Botulinum Research Coordinating Committee Meeting, November 2010 in Atlanta, GA.

This poster is available at <http://www.listlabs.com/posters.php>

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

It is recommended to reconstitute BoNT/A holotoxin with the reduction buffer, 20 mM HEPES, pH 8.0, containing 5 mM DTT, 0.3 mM ZnSO<sub>4</sub> 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 0.1% TWEEN 20 in the reduction buffer is essential for recovery of BoNT/A from the vial.

The reaction buffer for the CONTROL PEPTIDE for SNAPtide® 520 with BoNT/A holotoxin is the same buffer that is used for hydrolysis of SNAPtide® Prod #520 by BoNT/A holotoxin (20 mM HEPES, pH 8.0, containing 0.75 mM ZnSO<sub>4</sub>, 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 reaction with CONTROL PEPTIDE for SNAPtide® 520 with BoNT/A Light Chain, use the buffer for hydrolysis of SNAPtide® Prod #520, 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 TWEEN 20 or 1 mg/mL BSA is beneficial to the stability and storage of BoNT/A Light Chain at -20°C.

#### **Related Products:**

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

**Product #529:** UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 520. This is 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.

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

**Product #528:** UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 521. This is 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 #523:** SNAPtide® fIP6 containing a DABCYL/5-IAF FRET pair (U.S. Patent Pending #61/252,675).

For further information regarding this FRET peptide and related products, click on the posters tab on our website. [www.listlabs.com](http://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.

**FOR RESEARCH PURPOSES ONLY. NOT FOR USE IN HUMANS.**

Production: NS Date: 1/30/12 Management: NS Date: 1/30/12 QA/QC: CL Date: 1/30/12