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**CERTIFICATE OF ANALYSIS**  
 Unquenched Calibration Peptide for SNAP Etide® Prod #550  
 Lot #5591A1

**Contents**

Each vial of Unquenched Calibration Peptide for SNAP Etide® Prod #550 contains 50 nmoles of lyophilized peptide. 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). The peptide is used to generate a standard curve to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

**Purity**

The peptide is  $\geq 95\%$  pure as analyzed by reverse phase HPLC. The expected molecular weight was verified by mass spectrometry.

**Protocol for Standard Curve:**

The following protocol may be used to generate a standard curve using Prod #559. Use the same buffer, volume, temperature and excitation and emission settings as used in the SNAP Etide® cleavage assay. The excitation wavelength is 321nm with an emission at 418nm. Each dilution is read in triplicate using 250  $\mu\text{L}$ /well.

1. Make a 50  $\mu\text{M}$  stock solution of the calibration peptide by dissolving 1 vial in 2 x 500  $\mu\text{L}$ , for a total of 1000  $\mu\text{L}$ , of appropriate assay buffer. Cover with foil to protect from light. Store reconstituted peptide frozen at  $-20^{\circ}\text{C}$ .
2. Prepare a 5  $\mu\text{M}$  solution of the calibration peptide by diluting the 50  $\mu\text{M}$  stock solution 10-fold.
3. Make the following dilutions:

Final Concentration	5 $\mu\text{M}$ Calibration Peptide ( $\mu\text{L}$ )	Assay Buffer ( $\mu\text{L}$ )
1.2 $\mu\text{M}$ (0.300 nmoles)	240	760
1.0 $\mu\text{M}$ (0.250 nmoles)	200	800
0.8 $\mu\text{M}$ (0.200 nmoles)	160	840
0.6 $\mu\text{M}$ (0.150 nmoles)	120	880
0.4 $\mu\text{M}$ (0.100 nmoles)	80	920
0.2 $\mu\text{M}$ (0.050 nmoles)	40	960

(continued)

4. Add 250 mL of each dilution to the appropriate well of the microtiter plate. Each dilution is read in triplicate.
5. Immediately, place the microplate into the microplate reader and equilibrate for 5 minutes at the same temperature as the assay, prior to reading.
6. Plot the relative fluorescence unit versus amount of calibration peptide to obtain a calibration curve.

**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.

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

Production: ASZ

Date: 9-18-19

Management: WS

Date: 9-18-19

QA/QC: SW

Date: 9/18/19