



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

Contents

Each vial of Unquenched Calibration Peptide for SNAP Etide® Product #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 ≥ 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 Product #559. Use the same buffer, volume, temperature, and excitation and emission settings as used in the SNAP Etide® cleavage assay. The excitation wavelength is 321 nm with an emission at 418 nm. Each dilution is read in triplicate using 250 µL/well.

1. Make a 50 µM stock solution of the calibration peptide by dissolving 1 vial in 2 x 500 µL, for a total of 1000 µL, of appropriate assay buffer. Cover with foil to protect from light. Store reconstituted peptide frozen at -20°C.
2. Prepare a 5 µM solution of the calibration peptide by diluting the 50 µM stock solution 10-fold.
3. Make the following dilutions:

| Final Concentration | 5 µM Calibration Peptide (µL) | Assay Buffer (µL) |
|-----------------------|-------------------------------|-------------------|
| 1.2 µM (0.300 nmoles) | 240 | 760 |
| 1.0 µM (0.250 nmoles) | 200 | 800 |
| 0.8 µM (0.200 nmoles) | 160 | 840 |
| 0.6 µM (0.150 nmoles) | 120 | 880 |
| 0.4 µM (0.100 nmoles) | 80 | 920 |
| 0.2 µM (0.050 nmoles) | 40 | 960 |

4. Add 250 µL 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.

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Quality Assurance: *[Signature]* Date: 07 SEP 2021