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Product #528

CERTIFICATE OF ANALYSIS
UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 521
Lot #5281A2

**Contents:**

Each vial of the UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 521 contains approximately 50 nmoles of lyophilized peptide. The peptide content for this lot was determined by amino acid analysis. The average was 49.4 nmoles ± 3.0 with a %CV = 6. This peptide is identical to the cleavage product resulting from botulinum neurotoxin type A hydrolysis of the SNAPtide® (FITC/DABCYL), Prod #521 substrate. It contains the N-terminally-linked fluorophore, FITC. The peptide is used to generate a standard curve to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

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 lyophilized peptide. Cover the vial with foil to protect from light.

**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 #528. Use the same buffer, volume, temperature and excitation and emission settings as used in the SNAPtide® cleavage assay. The excitation wavelength is 490 nm with an emission at 523 nm. A cutoff filter at 495 nm was used. Each dilution is read in triplicate using 250 µl/well.

1. Make a 0.5 mM stock solution of the calibration peptide by dissolving 1 vial (49.4 nmoles) in 98.8 µl of DMSO. Cover with foil to protect from light and store frozen at -20°C.

2. On the day of the assay, prepare a 1 µM solution of the calibration peptide in the assay buffer.

3. Make the following dilutions:

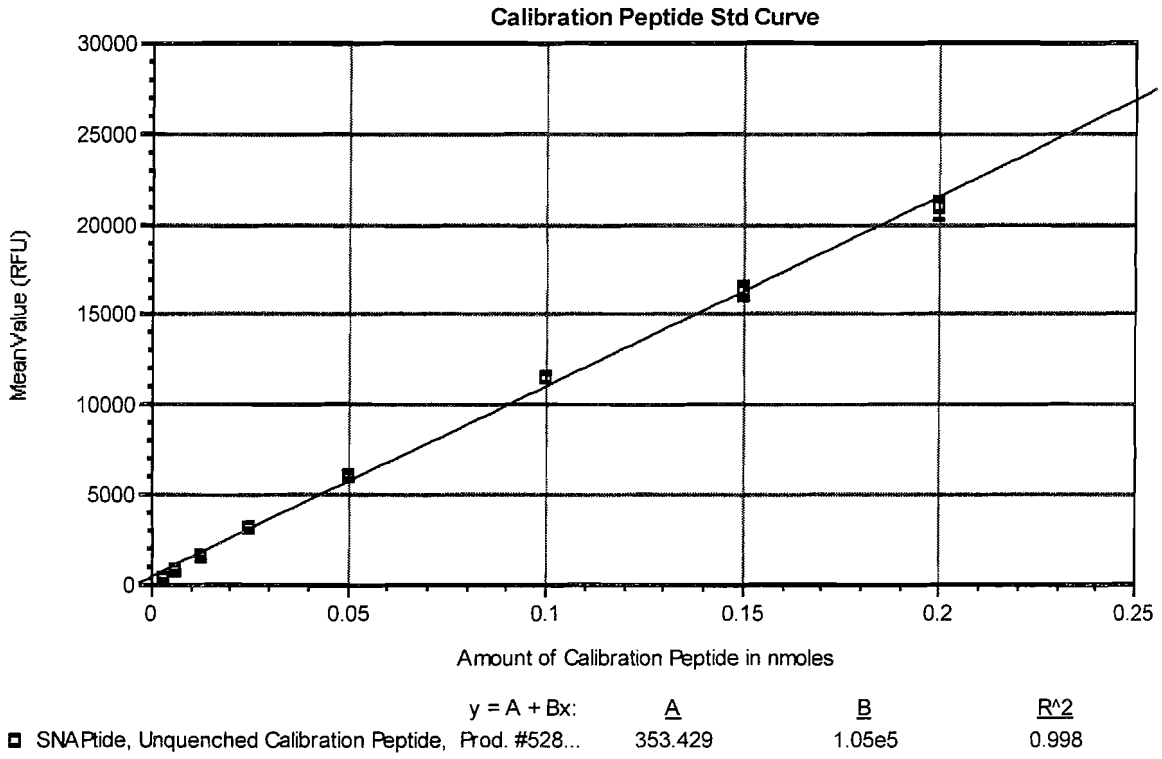
| Final concentration | µl of 1 µM Calibration Peptide | µl ASSAY BUFFER |
|---|---|---|
| 0.8 µM (0.200 nmoles) | 800 | 200 |
| 0.6 µM (0.150 nmoles) | 600 | 400 |
| 0.4 µM (0.10 nmoles) | 400 | 600 |
| 0.2 µM (0.050 nmoles) | 200 | 800 |
| 0.1 µM (0.025 nmoles) | 100 | 900 |
| 0.05 µM (0.0125 nmoles) | 50 | 950 |
| 0.025 µM (0.0063 nmoles) | 25 | 975 |
| 0.0125 µM (0.0031 nmoles) | 10 | 990 |
| 0 | 0 | 250/well |

(continued)

4. Add 250 µl of each dilution to the appropriate wells of the microtiter plate. Each dilution is read in triplicate.

5. Place the plate in the microplate reader and equilibrate for 15 minutes prior to reading.

6. A typical standard curve is shown here:




| | y = A + Bx: | A | B | R^2 |
|---|---|---|---|---|
| ◘ SNAPtide, Unquenched Calibration Peptide, | Prod. #528... | 353.429 | 1.05e5 | 0.998 |

**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: VFC Date: 1/25/12 Management: NS Date: 1/25/12 QA/QC: CL Date: 1/25/12