list labs
540 Division Street, Campbell, California 95008-6906
Tel. (408) 866-6363 Fax (408) 866-6364
www.listlabs.com

Product #529
Lot #5292A2

CERTIFICATE OF ANALYSIS
UNQUENCHED CALIBRATION PEPTIDE FOR SNAPTIDE® 520
Lot #5292A2

Contents

Each vial of the UNQUENCHED CALIBRATION PEPTIDE for SNAPtide® 520 contains approximately 50 nmoles of lyophilized peptide. The peptide content for this lot was determined by amino acid analysis. The average was 44.3 nmoles ± 0.5 with a %CV = 1.2. This peptide is identical to the cleavage product resulting from botulinum neurotoxin type A hydrolysis of the SNAPtide® (o-Abz/Dnp) Product #520 substrate. It contains the N-terminally-linked fluorophore, o-aminobenzoic acid (o-Abz). 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.

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 #529. Use the same buffer, volume, temperature, and excitation and emission settings as used in the SNAPtide® 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 (44.3 nmoles) in 886 µl of appropriate assay buffer. 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.

3. Make the following dilutions:

Final Concentration	µl of 1 µM Calibration Peptide	µl of 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	0	250/well

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

6. Plot the relative fluorescence units 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 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.

Quality Assurance: _____ Date: 14 JUL 2021