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Certificate of Testing

Unquenched Calibration Peptide for SNAPtide® 520

Prod. No: 529 Lot Number: 5292A3

Date of Manufacture 05 March 2026 **FOR RESEARCH PURPOSES ONLY.
NOT FOR HUMAN USE.**

Contents

Each vial of product 529, 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. 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, a-aminobenzoic acid (o-Abz). The peptide is used to generate a standard curve to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

Storage/Shipping Requirements

This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light. Ship material on blue ice.

Handling

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.

The product is intended for research purposes by qualified personnel. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

Attribute	Test Method ID / Version	Results	Pass / Fail
Appearance	Physical Appearance / ASSAY.063 v6.0	Before reconstitution: white, uniform pellet, free of foreign material. After reconstitution: clear, colorless, particulate-free, fully dissolved	N/A
Potency	Amino Acid Analysis (BioPharmaSpec)	47.1 nmoles/vial	N/A
Identity	Calibration Curve / ASSAY.196 v1.0	Vial to vial variability: %CV = 1.6% Lot to lot comparison: %CV = 9.4%	Pass

Quality Control: Date: 27 Mar 26
 Production: Date: 03/27/2026



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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 reconstituting a vial using an appropriate assay buffer with the appropriate volume (refer to page 1 for nmoles/vial results to determine reconstitution volume). 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 uM (0.200 nmoles)	800	200
0.6 uM (0.150 nmoles)	600	400
0.4 uM (0.10 nmoles)	400	600
0.2 uM (0.050 nmoles)	200	800
0.1 uM (0.025 nmoles)	100	900
0	0	250/well

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.