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Product #539

CERTIFICATE OF ANALYSIS
MAPKKide[®] , Unquenched Calibration Peptide for Product #530
Lot #5391A2

Contents:

Each vial of MAPKKide[®] , Unquenched Calibration Peptide contains 50 nmoles of lyophilized peptide. The peptide content for this lot was determined by amino acid analysis of 5 vials. The average was 52.2 nmoles \pm 1.1 with a %CV = 2.1. The solubility is at least 100 μ M in buffer, pH 8.2. Higher concentrations may be achieved in DMSO. This peptide is identical to the cleavage product resulting from Lethal Factor hydrolysis of the MAPKKide[®] substrate (product #530). 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.

Purity:

The peptide is >90% pure as determined by reverse phase HPLC. The expected molecular weight was verified by mass spectrometry.

Protocol for Standard Curve:

The following example protocol may be used to generate a standard curve using product #539. Use the same buffer, volume, temperature and excitation and emission settings as used in the MAPKKide[®] cleavage assay. In this example, the buffer is 20 mM HEPES, pH 8.2; the assay buffer used for cleavage of MAPKKide[®] by Lethal Factor. The plate reader is set to 37°C. The excitation wavelength is 321 nm with an emission at 418 nm. Each dilution is done in triplicate using 250 μ l/well.

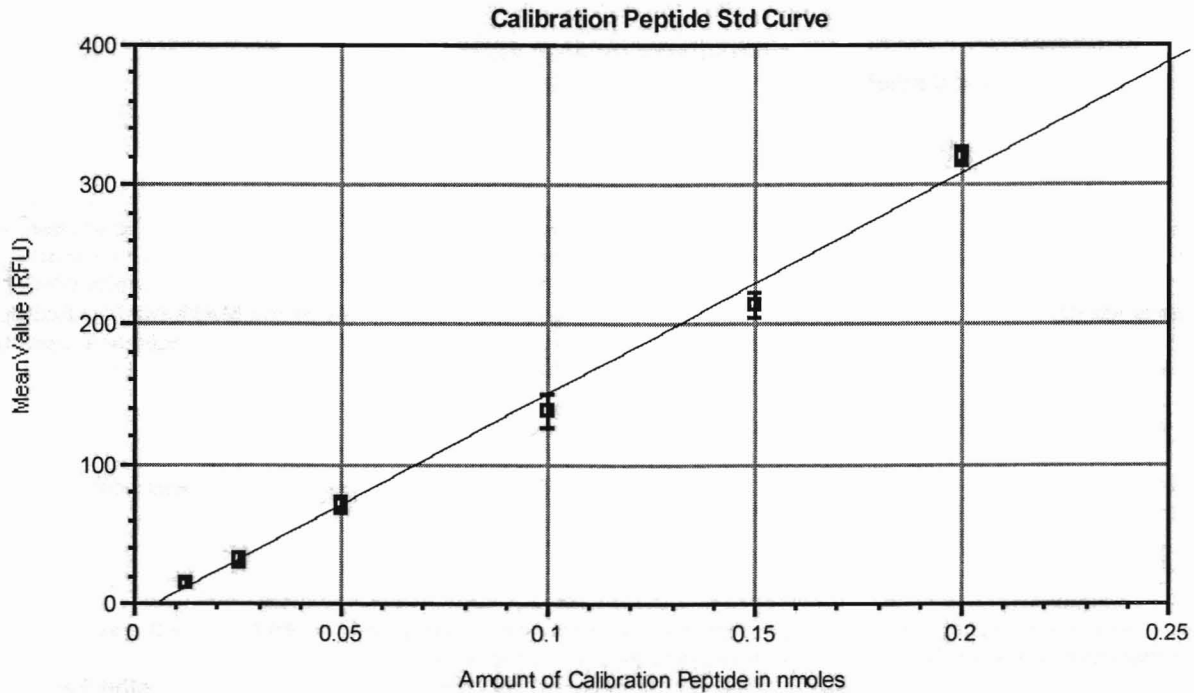
1. Make a 0.5 mM stock solution of the calibration peptide by dissolving 1 vial (52.2 nmoles) in 104.4 μ l of DMSO. Cover with foil to protect from light and store frozen.
2. On the day of the assay, prepare a 1.0 μ M solution of the calibration peptide in the assay buffer.
3. Make the following dilutions:

Final concentration	μ l of 1.0 μ M Calibration Peptide	μ l ASSAY BUFFER
0.8 μ M (0.200 nmoles)	800	200
0.6 μ M (0.150 nmoles)	600	400
0.4 μ M (0.10 nmoles)	400	600
0.2 μ M (0.050 nmoles)	200	800
0.1 μ M (0.025 nmoles)	100	900
0.05 μ M (0.0125 nmoles)	50	950
0	0	250/well

4. Add 250 μ l of each dilution to the appropriate well of the microtiter plate. Each dilution is done in triplicate.

(continued)

5. Place the plate in the microplate reader and equilibrate for 15 min prior to reading.
6. A typical curve is shown here.



	$y = A + Bx:$	<u>A</u>	<u>B</u>	<u>R²</u>
■ MAPKtide Unquenched Calibration Peptide, Prod. #539...		-9.872	1581.952	0.993

Packing/Storage:

This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light. After reconstitution, aliquot and store at -20°C.

Handling:

This product is not hazardous. Good laboratory technique should be employed in handling this product. This requires observing the following practices:

1. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.
2. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.
3. Avoid accidental autoinoculation by exercising care when handling in conjunction with any injection device.
4. 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 HUMAN USE.

Approved: NS Date: 3-18-10 Approved: Tc Date: 3/18/10