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Product #549

**CERTIFICATE OF ANALYSIS**
VAMPtide® Calibration Fluorophore for Product #540
Lot #5491A1

Contents:

Each vial of VAMPtide® Calibration Fluorophore contains approximately 100 nmoles of lyophilized compound. The exact content for each vial of this lot was determined by amino acid analysis of 4 vials. The average was 86.4 nmoles ± 0.9 with a %CV = 1.1. The solubility is at least ~300 µM in water. Higher concentrations may be achieved in DMSO. It is recommended that stock solutions be made in DMSO for dilution in buffer on day of use. This compound contains the fluorophore, o-aminobenzoic acid (o-Abz), which is linked to the ε-amino group of lysine. This compound is used to generate a standard curve to convert relative fluorescence units (RFU) to nmoles of cleaved substrate.

Purity:

The compound 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 #549. Use the same buffer, volume, temperature and excitation and emission settings as used in the VAMPtide® cleavage assay. In this example, the buffer is 20 mM HEPES, pH 7.4, 0.2% TWEEN-20, 5 mM DTT, 0.05 mM $ZnSO_4$; it is the same assay buffer used for cleavage of VAMPtide® by BTB . 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 µl/well.

1. Make a 50 µM stock solution of the calibration peptide by dissolving 1 vial (86.4 nmoles) in 1728 µl of assay buffer ( 20 mM HEPES, pH 7.4, 0.2% TWEEN-20, 5 mM DTT, 0.05 mM $ZnSO_4$). Then prepare 5 µM solution by diluting 50 µM solution 1:10, by adding 30 µl of 50 µM stock solution to 270 µl of assay buffer. Prepare fresh for use on the same day. Cover with foil to protect from light.

2. Make the following dilutions:

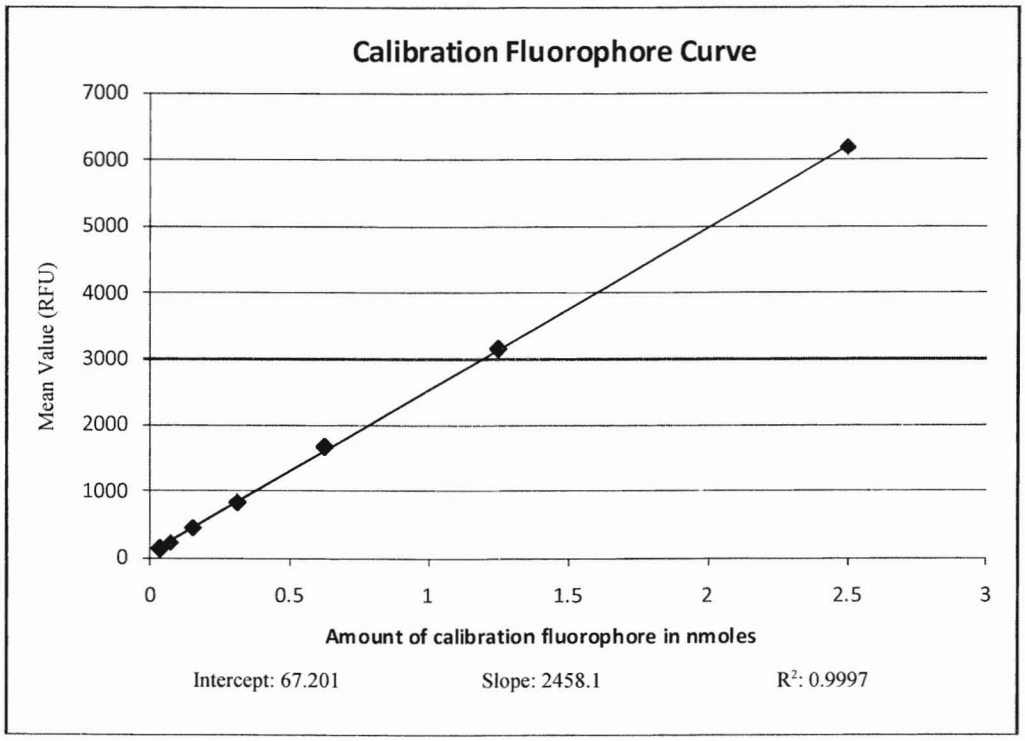
| **Final concentration** | **µl of 50 µM Calibration Fluorophore** | **µl ASSAY BUFFER** |
|---|---|---|
| 10 µM (2.5 nmoles) | 200 | 800 |
| 5 µM (1.25 nmoles) | 100 | 900 |
| 2.5 µM (0.625 nmoles) | 50 | 950 |
| 1.25 µM (0.313 nmoles) | 25 | 975 |
| **Final concentration** | **µl of 5 µM Calibration Fluorophore** | **µl ASSAY BUFFER** |
| 0.625 µM (0.156 nmoles) | 125 | 875 |
| 0.30 µM (0.075 nmoles) | 60 | 940 |
| 0.150 µM (0.38 nmoles) | 30 | 970 |
| 0 | 0 | 250/well |

3. Add 250 µl of each dilution to the appropriate well of the microtiter plate. Each dilution is done in triplicate.

(continued)

4. Place the plate in the microplate reader and equilibrate for 15 min prior to reading.

5. A typical curve is shown here.




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 throughly 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: EE Date: 05/27/09 Approved: NS Date: 05/27/09