Rapid and Sensitive Fluorescent Peptide Quantification Using LavaPep

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Purpose

- To develop a fast, sensitive and robust fluorescent-based assay, to quantify peptides that is compatible with downstream proteomics processes such as mass spectrometric peptide mass fingerprinting (PMF).

Methods

- LavaPep working reagent was added to peptide samples of interest in a 1:1 ratio.
- Samples and reagents were allowed to react for 1 hour before measuring fluorescence.
- Fluorescence was read by fluorescence imager (e.g. fluorimeter, 96-well plate reader, laser scanner) at an excitation wavelength of 510nm and an emission wavelength of 610nm.
- Quantification of peptides was performed by interpolation against a standard curve.
- Peptide quantification was validated by Amino Acid Analysis (AAA).
- PMF compatibility of peptides was assessed by MALDI-TOF MS.

Results

- The peptide assay enables <100 ng/ml of peptide to be quantified.
- A linear dynamic range of >4 orders of magnitude was observed.
- The assay method is quick, in that it only takes 1 hour.
- Assay is amendable to many platforms, such as fluorimeters, fluorescence plate readers, and laser scanners.
- LavaPep does not interfere with downstream proteomics processes, allowing mass spectrometric PMF of the same sample used for quantification.

Background

Peptide quantification is prerequisite in many areas of proteomics and peptidomic. For example, methods to separate peptides based on chromatography or electrophoresis, require accurate loading of peptides mixtures (e.g. from tryptic digests) to achieve the required sensitivity, without overloading. There are a number of colorimetric peptide assays such as ninhydrin, Lowry and BCA but these methods often lack the required sensitivity, particularly when samples are precious. AAA is often regarded as the 'gold standard' but the method is relatively expensive and is usually outsourced, making it inconvenient.

Epiccoconone,[2] is the fluorescent reagent that is the basis of the LavaPep Kit. It is a natural product that spontaneously conjugates to the amine of lysine and to a lesser degree arginine and histidine residues in proteins and peptides yielding an intensely fluorescent red product.[3] Covalent binding of LavaPep to proteins is stable in acidic environments (pH 2.4), therefore the fluorophore can be removed by changing pH. Reversible binding renders the peptides amenable to downstream proteomics analyses, such as mass spectrometry, Edman degradation, HPLC and functional assays.

Materials

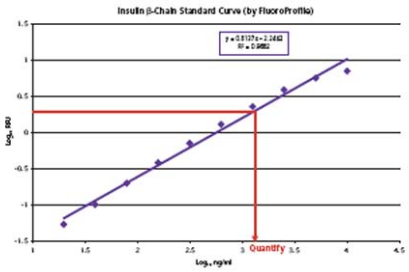
- LavaPep Protein Quantification Kit (Sigma-Aldrich Cat. No. FP0010)
- Peptides: - Insulin β–chain (Sigma-Aldrich Cat. No. 16383)
  - Fibronectin Fragment 137-1388 (His-Ser-Arg-Asn-Ser-Ile-Thr-Leu-Thr-Asn-Leu-Thr) (Sigma-Aldrich Cat.No. F0793)
  - Fibrinogen- γ Fragment 400-411 (His-His-Leu-Gly-Gly-Ala-Lys-Gin-Ala-Gly-Asp-Val) (Sigma-Aldrich Cat. No. F9145)
  - Gly-Arg-Ala-Asp-Ser-Pro-Lys (Sigma-Aldrich Cat. No. G4144)
  - Lys-Lys-Lys-Lys (Sigma-Aldrich Cat. No. L9026)

Methods (example)

1) Add Peptide
   Add LavaPep
   Working Reagent
2) Incubate 1 Hour

3) Measure Fluorescence
Ex/Em = 510/610 nm
Interpolate against
Standard Curve

4) Mass Spectrometric
PMF Compatibility



Results

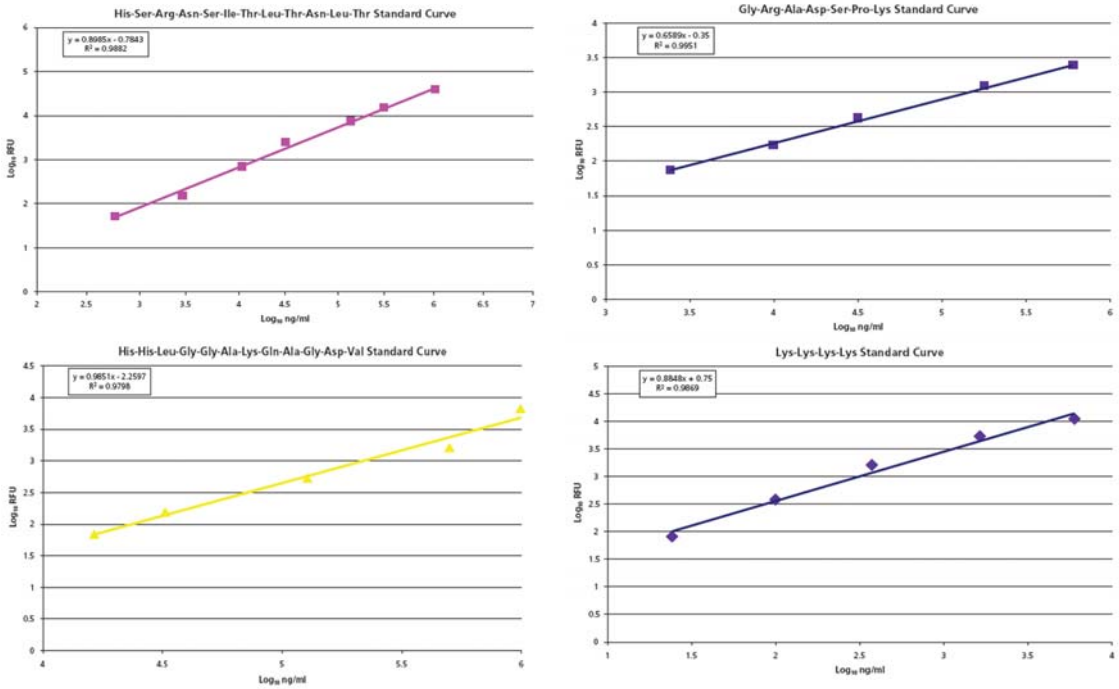
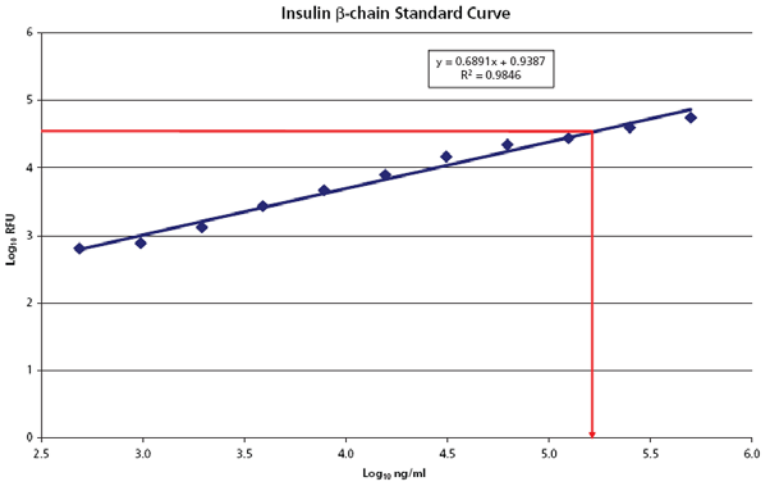


Figure 1: Various peptides were used to generate standard curves in order to determine the limitation of the LavaPep method for quantifying. These peptides included either Lys, Arg, or a combination of both Lys and Arg residues. As shown in the graphs, peptides with these residue types are able to generate useful standard curves for this assay. Other peptides, such as a Gly-Gly peptide, which contain no Lys or Arg residues did not show a change in fluorescence with a change in the peptide concentration (data not shown).



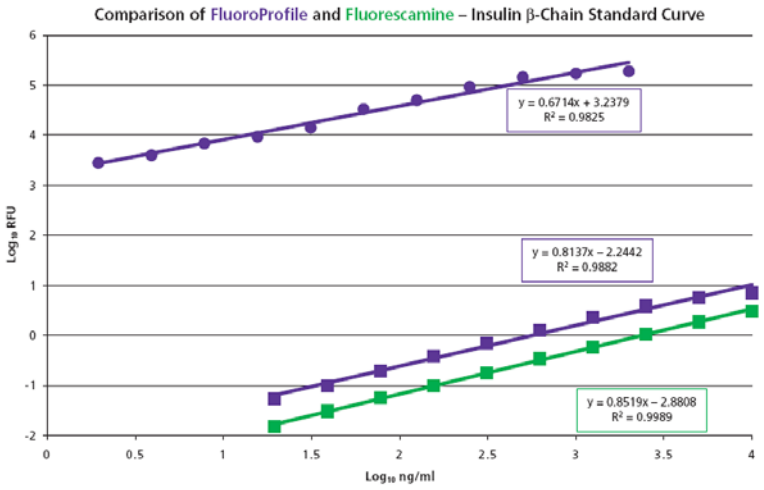
Peptide Quantification of Unknown Using the FluoroProfile Method

Figure 2: The LavaPep method is highly suitable for use in quantifying unknown peptide samples, such as insulin β–chain. A separately measured insulin β–chain peptide standard curve was used to quantify an unknown sample of insulin β–chain by interpolation against the standard curve. By this method, the unknown peptide sample had an apparent concentration of approximately 156μg/ml, as compared to the expected result of 147μg/ml by weight.

Peptide Sequence	Sigma-Aldrich Cat. No.	Concentration Determined by FluoroProfile	Concentration Determined by AAA
His-Ser-Arg-Asn-Ser-Ile-Thr-Leu-Thr-Asn-Leu-Thr	F0793	212 μg/ml	163 μg/ml
Gly-Arg-Ala-Asp-Ser-Pro-Lys	G4144	88 μg/ml	59 μg/ml
Lys-Lys-Lys-Lys	L9026	66 μg/ml	60 μg/ml
His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val	F9145	203 μg/ml	139 μg/ml

Validation of Quantification Results by AAA

Table 1: Results from the LavaPep method of quantifying peptides were validated by AAA peptide quantification. Various peptides, see list in table, were measured at several concentrations using both the LavaPep assay and AAA. The results were compared to show the validity of the LavaPep method. The LavaPep method is most useful for peptides containing lysine, arginine, and histidine residues.



Peptide Standard Curve

Figure 3: The LavaPep assay is capable of generating a peptide standard curve using peptides that contain lysine, arginine and/or histidine residues. The LavaPep method (blue) of obtaining a standard curve is compared to fluorescamine (green).[4] A sample of insulin β–chain peptide was dissolved in deionised water and serially diluted to generate a standard curve. Fluorescence from each assay methods was developed by excitation and emission at the appropriate wavelengths for each fluorophore. The linear range for each standard curve was calculated and plots of each were overlaid in the graph. The line of best fit was calculated. Wallac plate reader (●) SpectrMax plate reader (■).

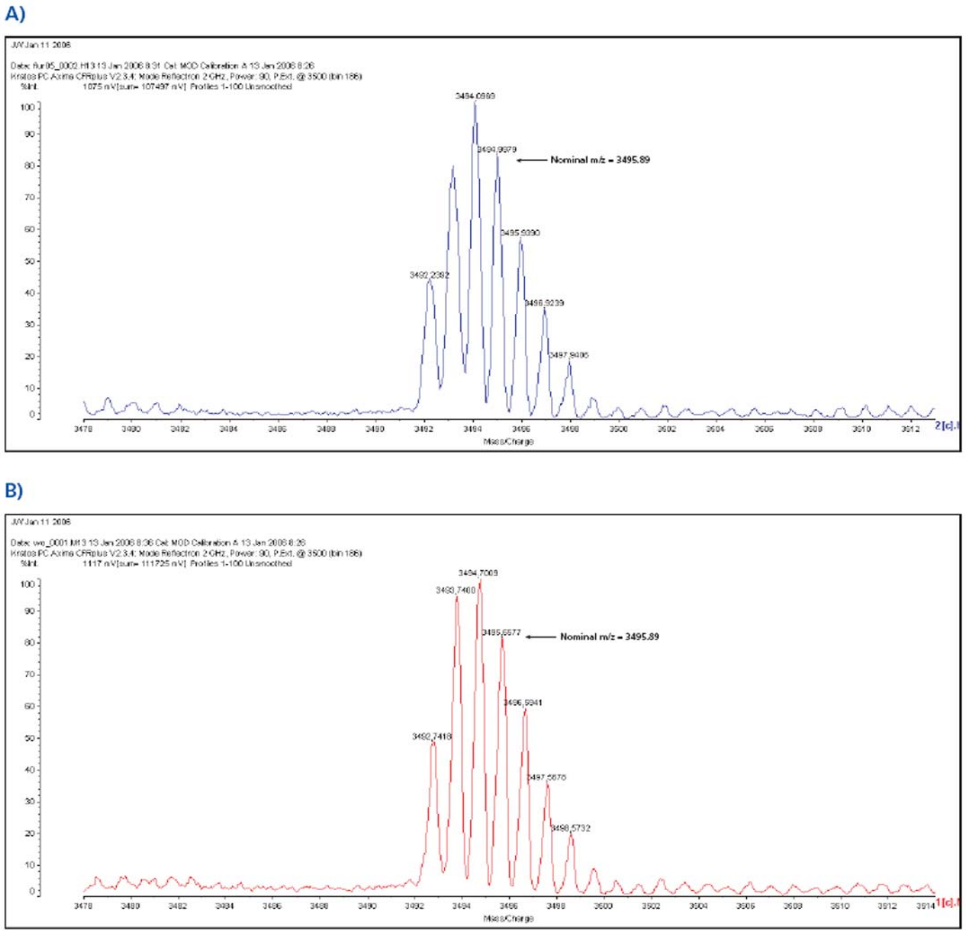


Figure 4: Importantly, the LavaPep reagent does not interfere with downstream analyses such as MALDI-TOF MS. As illustrated in the figure, essentially the same mass spectra were observed both with and without the addition of the LavaPep working reagent to the sample. Insulin β–chain peptide was first quantified using the LavaPep assay method. Then, the same sample was prepared and pplied to a MALDI target **(A)**. A similar sample, without the addition of the LavaPep working reagent, underwent the identical procedure **(B)**.

Conclusion

- LavaPep was found to be suitable for quantification of peptides containing lysine, arginine, and histidine residues.
- LavaPep does not derivatize the peptides, allowing assessment of the sample by MALDI-TOF MS or other downstream proteomic processes. Other peptide quantification methods employ one sample for quantification purpose and a separate sample for downstream applications.
- By using the same sample, as with the LavaPep reagent, two major benefits are obvious: 1) no need to 'waste' precious sample, and 2) quantification of the exact sample used in the downstream application, such as exemplified with the MALDI-TOF MS data.

References

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