Instructions for Capillary Electrophoresis Peptide Analysis Kit

Catalog Number 148-4110

BIO-RAD

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Section 1
Introduction

High performance capillary electrophoresis is a powerful technique for achieving high resolution separations of peptides. In many cases, peptides with small differences in molecular structure can be readily resolved, often in a matter of minutes.

The Peptide Analysis Kit provides a convenient tool for performing peptide separations with the BioFocus capillary electrophoresis systems. It includes all necessary reagents and a detailed analysis protocol. The kit is useful for a variety of applications including purity determination of synthetic peptides, separation of peptide digests, and characterization of fractions recovered from HPLC separations.

1.1 Components supplied in the kit

pH 2.5 Phosphate Buffer, 0.1 M, 250 ml, catalog number 148-5011

Peptide Calibration Set, catalog number 148-2012

Instructions for use, analysis protocol, document number, LIT-285.

1.2 Supplies needed

For use with the BioFocus system

BioFocus capillary cartridge, catalog number 148-3052
distilled or deionized water, filtered
disposable microcentrifuge tubes, 500 µl, catalog number 223-9503
BioCAP™ LPA coated capillary, 50 µm ID cut to 24 cm, catalog number 148-3070

Section 2
Analysis Protocol

This protocol assumes that the user has basic familiarity with the operation of the CE system to be used. For an introduction or review of operating procedures and the terminology, the user is referred to the system operating manual.

The Peptide Calibration Set is used to demonstrate the peptide analysis protocol, assess the operation of the instrument and test the performance of the capillary cartridge.

The calibration set contains a mixture of 25 µg each of the following nine peptides in lyophilized form: bradykinin, angiotensin II, α-melanocyte stimulating hormone, thyrotropin releasing hormone, luteinizing hormone-releasing

hormone, leucine enkephalin, bombesin, methionine enkephalin, and oxytocin. After proper dilution, the final concentration of each peptide is 50 µg/ml.

The calibrator peptides are reconstituted by adding 0.5 ml of a 1:9 V/V dilution of phosphate buffer (0.1 M phosphate buffer, pH 2.5, catalog number 148-5011 or 148-5010) with distilled water. After reconstitution, refrigeration at 4 °C is recommended.

The best peptide separations are usually achieved under acidic conditions, typically below pH 3. The electrolyte supplied with this kit is 0.1 M phosphate buffer, pH 2.5.

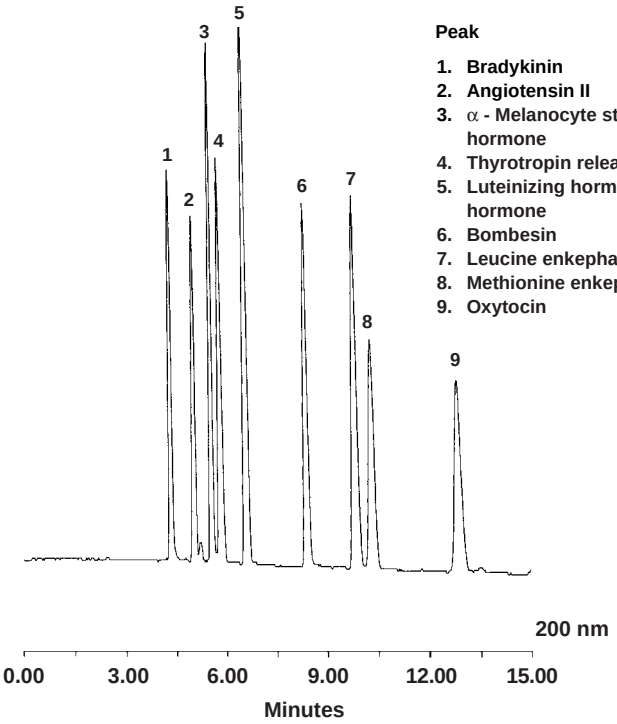
Section 3
Instruction For Use of the pH 2.5 Phosphate Buffer

3.1 Use with the BioFocus system

1. Reconstitute the Peptide Calibration Set as instructed and place a minimum of 20 µl in a 500 µl microcentrifuge tube.

2. The following parameters should be incorporated into the method for running this standard:

Method type:	CZE
Operating mode:	Constant voltage
Polarity setting:	Positive-to-negative
Running voltage:	10 kV
Current limit:	100 µA
Cartridge temperature:	25 °C
Carousel compartment temperature:	20 °C
Sample injection:	Electrophoretic, 8 kV for 8 seconds
Preparation cycle:	Phosphate Buffer (catalog number 148-5010 or 148-5011) for 20 seconds at high pressure
Detector mode:	Single wavelength
Wavelength:	200 nm
AU range:	0.02 AUFS
Running time (stop time):	15 minutes
Rise time:	1 second



Peak		MW
1.	Bradykinin	1,060
2.	Angiotensin II	1,046
3.	α - Melanocyte stimulating hormone	1,665
4.	Thyrotropin releasing hormone	362
5.	Luteinizing hormone releasing hormone	1,182
6.	Bombesin	1,620
7.	Leucine enkephalin	392
8.	Methionine enkephalin	574
9.	Oxytocin	1,007

Fig. 1. Electropherogram of Peptide Calibration Set, catalog number 148-2012.

Section 4
Sample Preparation

4.1 Sample Concentration

For the best peak detection and quantitation (signal-to-noise>5), the sample concentration should be at least 1 µg/ml per peptide. For analysis of dilute samples, preconcentration may be necessary. Although preconcentration by evaporation or lyophilization can be used, these procedures will result in an increase in the sample salt concentration as well. The presence of salt in the sample will reduce sensitivity and efficiency when using electrophoretic injection. For adequate sensitivity, the sample salt concentration should be 0.01 M or less.

4.2 Desalting

For samples containing higher concentrations of salt, a desalting step will be required to achieve good sensitivity. Conventional desalting methods such as dialysis, gel filtration, and ultrafiltration work well for large polypeptides (>10,000 M_r) but are unsatisfactory for smaller peptides.

For smaller peptides, desalting using hydrophobic sorbents is often effective. These sorbents (usually alkyl hydrocarbons bonded to microparticulate silica) are available in bulk form or packed into disposable cartridges. An aqueous

peptide sample is adsorbed onto the material, salts are washed away with water, and peptides are then eluted in a small volume using an aqueous:organic eluant such as water:acetonitrile. Recovery of most peptides is good, although small hydrophilic peptides may be lost due to weak binding to the sorbent.

Section 5
Optimization of Peptide Separations

The phosphate buffer supplied in this kit is formulated to give good resolution of a wide range of peptides using the 50 µm inside diameter coated capillary cartridges with the protocol outlined above. In cases where increased resolution or sensitivity is desired, the following guidelines should be considered.

5.1 Increasing Resolution

Resolution can be increased by increasing the capillary length. However, analysis times will be longer due to the increased capillary length. To maintain the same field strength, the operating voltage can be increased.

Resolution also depends upon the width of the sample zone injected into the capillary; narrow zones improve resolution. Narrow sample zones can be obtained by reducing the injection time and/or the injection voltage for electrophoretic injection, or reducing the psi∗second value for pressure injection.

5.2 Increasing Sensitivity

The amplitude of the detector signal will depend upon the amount of sample introduced into the capillary during sample loading. This can be increased by raising the injection voltage, increasing the injection time, or increasing the psi* second value. Practical limits for electrophoretic injection are approximately 10 kV and 15 seconds, above which sensitivity is not significantly increased. For pressure injection, limits are dependent on the viscosity of the sample solution and running buffer. Remember that wider sample zones will also reduce resolution.

Higher sensitivity can also be achieved by reducing the sample salt concentration, which produces narrow, concentrated sample zones. Increasing the peptide concentration in the sample solution can also be used, but the concentration method must be one which does not also increase the salt concentration.

Sensitivity can be increased by selecting the optimal detection parameters. Peptides are typically detected at 200 nm, as this wavelength usually gives the highest absorbance signal. However, if the peptide of interest absorbs strongly at a longer wavelength, the wavelength setting on the detector can be reset to the appropriate value. Use of a larger inside diameter capillary will also increase sensitivity.

5.3 Storage Conditions

BioFocus Cartridge - To maximize the life of the coating, the following steps should be followed before storing the cartridge. A run group should be scheduled at the end of the automation sequence that cleans and prepares the capillary for storage. This run group should employ a method that includes the following purges:

Prep Cycle 1 - water for 60 seconds at high pressure

Prep Cycle 2 - nitrogen for 120 seconds at high pressure

Buffers - All buffers should be capped tightly and stored at 4 °C when not in use.

Section 6
Product Information

Catalog Number	Product Description
148-4110	**Capillary Electrophoresis Peptide Analysis Kit,** containing buffer, peptide calibration set and analysis protocol
148-3052	**BioFocus Capillary Cartridge**
148-3070	**BioCAP LPA Coated Capillary,** 50 µm ID
148-2012	**Peptide Calibrator Set**
148-5010	**Phosphate Buffer,** 0.1 M, pH 2.5, 60 ml x 4
148-5011	**Phosphate Buffer,** 0.1 M, pH 2.5, 250 ml

Section 7
References

1. Zhu, M., Hansen, D. L., Burd, S. and Gannon, F., *J. Chromatography*, **480**, 311 (1989).
2. Bio-Rad HPE application note 1575-13 “Measuring Degradation of Angiotensin.”
3. Bio-Rad Bulletin 1480 “Use of High Performance Electrophoresis in Peptide Analysis and Peptide Mapping.”
4. Bio-Rad Bulletin 1482 “Use of High Performance Electrophoresis in Monitoring Peptide Synthesis.”

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SIG 020996 Printed in USA

LIT285 Rev B