

Microvolume Spectrophotometric and Fluorometric Determination of Protein Concentration

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ABSTRACT

Methods for determining protein concentration that use progressively smaller amounts of material are continually being developed. A new way of minimizing the amount of sample used for spectroscopic analysis is providing more opportunities for greater quality control. Traditional spectrophotometric and fluorometric methods for determination of protein concentrations have long required placing samples into containment devices such as cuvettes or capillaries. A microsample retention system is changing that paradigm by using natural surface tension properties to capture and hold microvolume samples in place during measurement without traditional containment devices. The advantage of such a system is to dramatically reduce the amount of sample required (1 to 2 μl) while greatly increasing the dynamic range of protein concentrations that can be measured. Modifications to classic protein concentration determination protocols are presented to provide a microvolume alternative to traditional cuvette-based methods. *Curr. Protoc. Protein Sci.* 55:3.10.1-3.10.16. © 2009 by John Wiley & Sons, Inc.

Keywords: microvolume • microvolume spectrophotometer • microvolume fluorospectrometer • microsample • microvolume spectroscopy • protein concentration • microsample analysis • microvolume analysis

INTRODUCTION

Advances in photonic technologies enable investigators to perform spectroscopic analyses of progressively smaller amounts of isolated sample. Classical methods of spectrophotometry and fluorometry require containment devices such as cuvettes and capillaries. In many cases, limited amounts of analyte material often preclude the use of traditional spectrophotometers and fluorometers. Microsample spectroscopy circumvents these issues by capturing microvolume samples between two measurement surfaces using the inherent surface tension of the sample, greatly reducing the amount of sample consumed during the measurement. The protocols in this unit provide microvolume sample quantitation alternatives to classical methods of spectroscopic protein quantitation, described thoroughly in *UNIT 3.4*.

Basic Protocol 1 is a microvolume protocol for traditional protein measurements performed directly using the absorbance reading at A_{280} . Basic Protocol 2 describes a modified BCA method as an example of how to perform microvolume protein quantitation using a colorimetric assay. Basic Protocol 3 describes the Quant-iT dye fluorescence assay as an example of how to perform microvolume protein quantitation using a common fluorophore.

MICROVOLUME SPECTROPHOTOMETRIC DETERMINATION OF PROTEIN CONCENTRATION USING A_{280} ABSORBANCE

Absorbance analysis of limited-volume samples has become of paramount importance as more molecular biology techniques require accurate quantitation of proteins with minimal

BASIC PROTOCOL 1

Detection and Assay Methods

3.10.1

Supplement 55

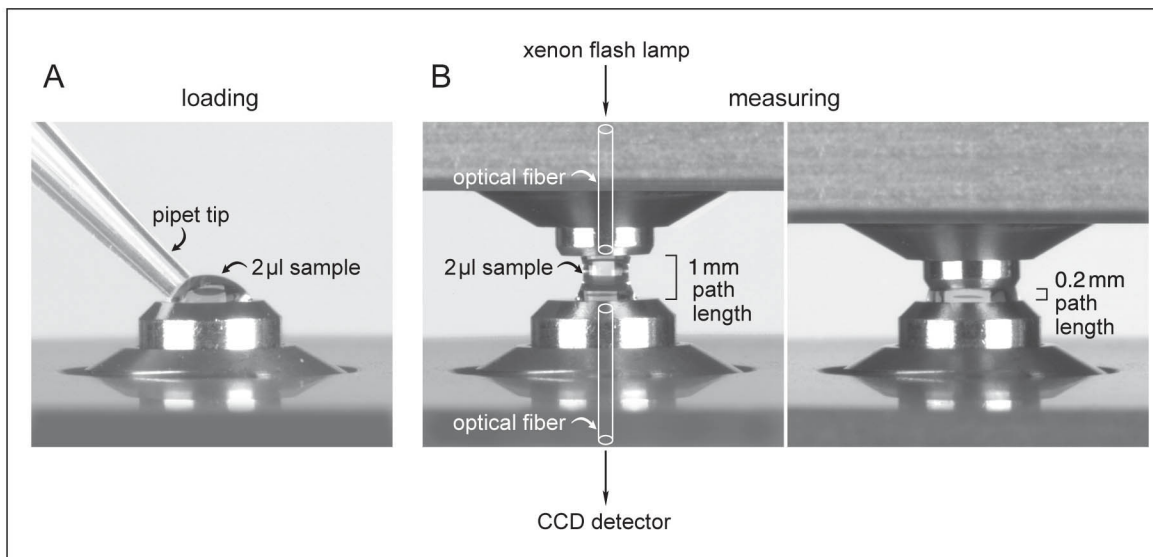


Figure 3.10.1 The NanoDrop 1000 spectrophotometer sample retention system. **(A)** A sample volume of 2 μl is dispensed onto the lower optical pedestal. **(B)** Once the instrument lever arm is lowered, the upper optical pedestal engages the sample, forming a liquid column with the path length defined by the gap between the two optical surfaces. During each measurement, the sample is assessed at both the 1-mm and 0.2-mm path length, providing a wide concentration range for protein quantification.

Table 3.10.1 Concentration Ranges of Bovine Serum Albumin (BSA) Suitable for Traditional 1-cm Path Length Cuvettes in a Traditional Spectrophotometer and for a Variable Path Length (1 mm to 0.2 mm) Microsample Spectrophotometer

Property	Single- or dual-beam cuvette-based spectrophotometer (1.0-cm path length)	Microsample spectrophotometer (0.2- to 1.0-mm path length)
Sensitivity	~ 0.001 mg/ml	0.1 mg/ml
Maximum concentration	~ 4 mg/ml	100 mg/ml

sample consumption. Many protein purification and isolation techniques produce a very limited sample volume; thus conventional spectrophotometric methods are impractical or impossible. The classic approach to address the problem of sample being wasted for quality control spectroscopic measurements was to reduce the volume of the sample container (small volume cuvettes, capillaries). The NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific) solves this problem by employing a sample retention system that combines fiber optic technology and the inherent surface tension properties of liquid samples to retain microsamples during measurement without the use of cuvettes or capillaries. This microvolume spectrophotometric instrument can accurately quantify proteins in volumes as small as 2 μl . The sample is coupled directly to optical surfaces where surface tension holds it in place during the measurement cycle (Fig. 3.10.1).

The absence of a sample containment device reduces the inherent absorbance difference in cuvettes and capillaries, and allows changes in path length to occur in real time. Changing the path length in real time dramatically reduces the total measurement time and greatly broadens the range of concentrations that can be measured. During each measurement cycle, the sample is assessed at both a 1- and 0.2-mm path length, providing an extensive concentration measurement range (0.1 to 100 mg/ml for purified BSA; Table 3.10.1). The Beer-Lambert Law correlates absorbance of a chromophore, such as certain