EZQ® Protein Quantitation Kit (R33200)

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light

Ex/Em: 280 and 450/618 nm

Number of Assays: 2000

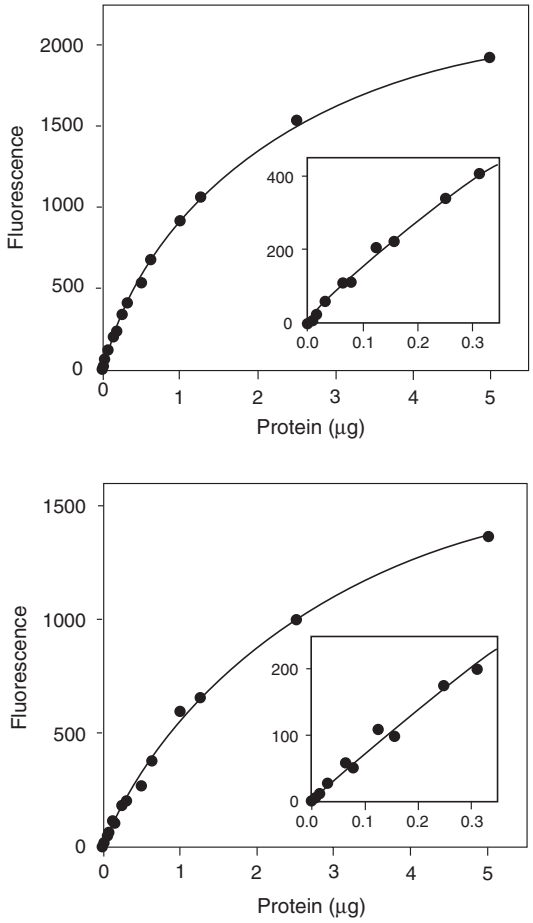

Figure 1. EZQ® assay of ovalbumin. A dilution series of ovalbumin was prepared, assayed as described in the text and then quantitated using both a 473 nm laser-based scanning instrument (upper panel) and a fluorescence microplate reader (lower panel). The assays were performed over a broad range, and the insets show the low range in greater detail. The assays were performed in triplicate, and the mean values, in arbitrary fluorescence units, were plotted after subtracting background values of 86 (upper panel) or 18 (lower panel).

Introduction

The EZQ® Protein Quantitation Kit provides a fast and simple assay for proteins in solution. Because detergents, reducing agents, urea, and tracking dyes do not interfere, this fluorescence-based quantitation kit is ideal for determining protein concentrations of samples prior to polyacrylamide gel electrophoresis. The protein assay requires only 1 µL of sample, and up to 96 samples, including standards, can be assayed in one session. In the assay, the protein samples are spotted onto one of the provided assay papers, fixed onto the paper and then stained with our proprietary EZQ® protein quantitation reagent. After spotting the samples, completing the protocol takes only about 1 hour. The protein concentration is determined from a standard curve, and the effective range for the assay is generally 0.02–5 mg/mL, or 0.02–5 µg per spot (Figure 1). Protein-to-protein sensitivity differences in the assay are minimal—the observed coefficient of variation is ~16% (Figure 2).

The EZQ® Protein Quantitation Kit is designed for high-throughput analysis. The solid-phase format and special EZQ® 96-well microplate cassette can be used with readily available fluorescence-detection instruments, for example:

- Fluorescence microplate readers, reading either from the top or bottom of the plate
- Laser-based scanning instruments, equipped with 450, 473 or 488 nm lasers
- UV illuminators in combination with photographic or CCD cameras for image documentation and analysis

Materials

Contents

- **EZQ® protein quantitation reagent** (Component A), 1 L
- **EZQ® 96-well microplate cassette** (Component B)
- **Assay paper** (Component C), 25 sheets
- **Ovalbumin (chicken egg) for preparing protein standards** (Component D), 2 vials, each containing 2.0 mg

Storage

Upon receipt, store the EZQ® Protein Quantitation Kit at room temperature, protected from the light. The kit components should be stable for at least 6 months.

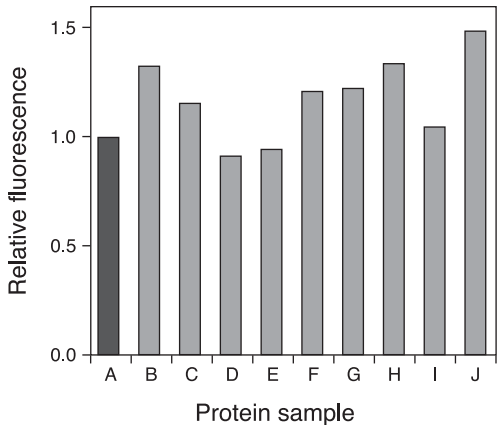

Figure 2. Protein-to-protein variation in the EZQ® assay. Triplicate 1 µg samples of various proteins were assayed using a fluorescence-based microplate reader as described in the text. The mean fluorescence values, after correcting for background fluorescence, are expressed relative to that of ovalbumin. The coefficient of variation is 16%. The protein samples are: A) ovalbumin; B) bovine serum albumin; C) myoglobin; D) soybean trypsin inhibitor; E) β-casein; F) carbonic anhydrase; G) transferrin; H) mouse IgG; I) lysozyme; and J) histone.

Materials Required but Not Provided

- Methanol
- Plastic staining tray
- Rinse buffer (10% methanol, 7% acetic acid)

Protocol

Prepare the Protein Standards

1.1 Make a stock solution of ovalbumin. The ovalbumin (Component D) supplied with the kit can be used to make protein standards for the assay. To make a 10 mg/mL stock solution, add 200 µL of buffer to one vial containing 2.0 mg of ovalbumin, and mix well. The buffer used should be the same as that used for the experimental samples. Dispense aliquots of the stock solution into microfuge tubes and store at ≤–20°C for future use.

1.2 Prepare dilutions of the ovalbumin stock solution. Prepare standards by making serial dilutions of the 10 mg/mL ovalbumin stock solution. The dilution buffer should be the same as that used for the experimental samples. Denaturing buffers containing dithiothreitol or TCEP are recommended (see below). At least five concentrations should be used to cover the range expected for the experimental samples. The full effective protein concentration range for this assay is ~0.02–5 mg/mL. Volumes of 1 µL are used in the assay.

Prepare the EZQ® 96-well Microplate Cassette

2.1 Insert the assay paper. Place the microplate face down on a clean surface. Wearing gloves, place a sheet of assay paper (Component D) over the microplate, and align the paper with the inner tabs of the top, bottom, and left sides of the plate. Mark one corner of the paper with a pencil to identify the orientation. If desired, assay papers can be cut in half and used in the device.

2.2 Insert the backing plate. To insert the stainless steel backing plate into the microplate, hold the backing plate so that the flexible bar is at the top. Place the bottom tabs of the backing plate along the bottom, inner edge of the microplate. Apply gentle pressure to the flexible bar and guide the top edge of the plate into position. The top tabs should fit into the top, inner edge of the microplate. Release the pressure on the flexible bar and check that the paper is securely in place.

Note: To ensure a tight fit, the width of the opening beneath the flexible bar should not be narrower than 2 mm before inserting the stainless steel backing plate into the microplate. The opening can be manually adjusted to this width if required.

Load the Protein Standards and Samples

For the following procedure, the concentration of the protein samples should not exceed 5 mg/mL. If the concentration is too high, the samples can be diluted in the same buffer. Denaturing buffers containing dithiothreitol or TCEP are recommended. Ampholytes, up to a concentration of 1%, can be included in the sample buffers. If ampholytes are included in the experimental samples, be sure to include them in the protein-standard samples as well. If using ampholyte concentrations higher than 1%, then add them to the samples *after* the protein assay has been completed.

3.1 Spot the protein samples onto the assay paper. Protein samples can be applied to the assay paper either through the top wells of the cassette or through the backing plate on the bottom. Apply a 1 µL volume of each protein standard (prepared in step 1.2) and each experimental sample to separate wells of the microplate assembly. Include a 1 µL sample of buffer alone, to serve as a no-protein control. Loading each sample in triplicate is recommended. Be careful not to puncture or scratch the membrane with the pipet tip. Gently dispense samples from the pipet tip onto the paper without touching the pipet tip to the paper. Once in contact with the paper, the sample should wick out from the tip. Pipetting accuracy can be improved by gently wiping away any sample on the outside of the pipet tip before spotting the sample onto the paper.

3.2 Dry the samples. Allow the protein samples on the paper to dry completely. A hair dryer can be used to reduce the drying time to about a minute.

Stain the Protein Standards and Samples

4.1 Remove the assay paper from the cassette. Wearing gloves, remove the assay paper from the cassette by depressing the spring arm of the backing plate and tilting the backing plate up and away from the assay paper. Remove the protein-spotted assay paper.

4.2 Fix and wash the protein spots. Pour ~40 mL of methanol into a plastic staining tray. Use a plastic tray slightly larger than the assay paper. If half sheets of paper are used, use ~20 mL and a proportionally smaller tray. Place the protein-spotted assay paper into the methanol and wash, with gentle agitation, for 5 minutes. This step removes contaminating substances including urea, SDS, reducing agents, salts and dyes that may be present.

4.3 Dry the protein-spotted assay paper. After washing, air dry the assay paper, using a hair dryer, if desired.

4.4 Stain the proteins. Pour 40 mL of the EZQ® protein quantitation reagent (Component A) into a staining tray. Place the protein-spotted assay paper into the stain solution and agitate gently on an orbital shaker for 30 minutes. For half sheets of paper, use ~20 mL and a smaller staining tray.

4.5 Rinse the assay paper. After staining, rinse the assay paper for 1–2 minutes in rinse buffer (10% methanol, 7% acetic acid). Repeat twice, for a total of three rinses.

4.6 Prepare the protein-spotted assay paper for analysis. If protein spots will be analyzed using a laser-based scanner or UV illuminator, wet or dry assay papers can be used. If the protein spots are to be analyzed using a fluorescence-based microplate reader, the assay paper must be dry. Air dry the assay paper on a clean, flat surface, using a hair dryer, if desired.

Read and Analyze the Results

5.1 Read the fluorescence. EZQ® protein quantitation reagent has two excitation maxima, one at ~280 nm and another at 450 nm. The emission maximum is at ~618 nm. Various instruments can be used to read the fluorescence, as described below.

Microplate readers: Place the dried paper (from step 4.6) back into the microplate and secure the backing plate. A hand-held UV light can be used to ensure the correct alignment of the paper to the wells. Analyze the stained protein spots in a fluorescence-based microplate reader using excitation/emission settings of ~485/590 nm. Top- or bottom-reading microplate readers may be used, and it makes no difference whether the protein samples were spotted from the top or from the bottom. For optimal results, program the microplate reader to take multiple samplings or multiple readings of each well.

Note: The stainless steel backing plate must be securely in place before placing the cassette into the plate reader. If it is not secure, there is risk of jamming the instrument. Refer to step 2.2 to ensure proper adjustment. If desired, the backing plate may be taped into place using tape that does not autofluoresce.

Laser-based scanners: Image the stained assay paper using an imaging system equipped with a 450, 473 or 488 nm laser and an appropriate emission filter (e.g., a 520 or 580 nm longpass filter, or a 600 nm bandpass filter).

UV illuminators: To visualize the stained protein spots, illuminate the assay paper with a 300 nm transilluminator, a UV top-illuminating system or a hand-held UV-B light source. Use a photographic or CCD camera, with an appropriate filter, to generate digital images of the stained paper, and then quantitate the image with appropriate software.

5.2 Analyze the results. Calculate the fluorescence values of the experimental samples and standards by subtracting the fluorescence value of the no-protein control. Create a standard curve (see Figure 1) by plotting the corrected fluorescence values of the standards vs. the corresponding protein mass (or concentration). Determine the mass (or concentration) of the experimental samples from the standard curve.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
R33200	EZQ® Protein Quantitation Kit *2000 assays*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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