

Protein Quantitation Using the Quantify™ Protein Assay System

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Promega's Quantify™ Protein Assay System provides a fast, sensitive method for the quantitation of proteins in solution in a convenient 96 well format. Unlike traditional quantitation methods, the Quantify™ Assay can detect as little as 200ng of total protein and is compatible with reagents that usually interfere with protein quantitation, including Promega's Cell Culture Lysis Reagent and Reporter Lysis Buffer.

Introduction

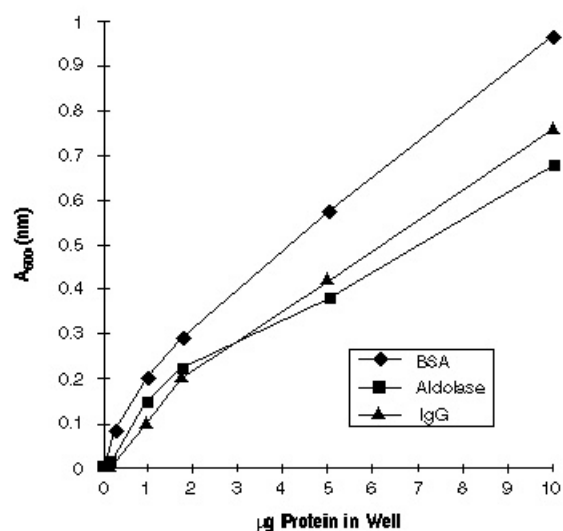
The study of many biochemical processes depends upon an accurate measurement of the amount of protein in solution. This need to accurately determine the concentration of protein has led to the development of several protein quantitation methods. One of the most widely used methods was developed by Bradford (1), which is based upon a shift in the absorption spectra of Coomassie® Brilliant Blue G-250 when the dye binds to protein in an acidic solution. The method is fast, convenient, and produces an equivalent absorbance change for many different proteins. The Bradford assay has been commercialized by several corporations as a liquid dye solution that is mixed with a protein sample. The absorbance of the resulting solution is measured spectrophotometrically at 600nm and compared to a standard curve generated using known quantities of a control protein in the dye solution.

As science turns to the study of many relatively rare cell proteins, the need has arisen to quantitate smaller and smaller amounts of protein in increasingly dilute solutions. One limitation of the Bradford method is that amounts of protein below 1µg are not easily detected because protein samples undergo a substantial amount of dilution during the assay. The Quantify™ Protein Assay System can easily quantitate amounts of protein less than 1µg because protein samples are not diluted during the assay. In addition, the Quantify™ System offers other advantages over traditional quantitation methods including a convenient 96 well format, and increased tolerance to agents which normally interfere in a Bradford reaction.

The Quantify™ protein assay system

The Quantify™ Protein Assay Reagent is a modified Bradford reagent which is provided in a lyophilized form in a 96 well plate. This format enables the user to process a large number of samples in a relatively short period of time. The protocol involves the simple addition of the protein-containing solution to the well, followed by detection of color in a microwell plate reader at a wavelength between 580-630nm. The predispensed, lyophilized reagent is provided in a vacuum-sealed bag and has a shelf-life of at least one year. Opened plates can be stored at room temperature for use withing one month.

The Quantify™ Reagent readily determines the concentration of very dilute protein samples. The system gives a linear response for protein amounts in the well from 200ng to 10µg, which corresponds to protein concentrations of 1-50µg/ml because the volume of the sample is only 200µl (Figure 1). Samples with protein concentrations greater than 50µg/ml do produce additional color, however the relative response is no longer linear.



1.

Figure 1. Total protein (μg) vs absorbance (A_{600}) for three different proteins. The graph shows the linear range of the Quantify™ Protein Assay System for bovine serum albumin (BSA), aldolase, and rat immunoglobulin (IgG).

Variability has been seen in the Quantify™ dye response to some proteins, similar to differences obtained using Bradford's reagent. For example, data in [Figure 1](#) shows approximately a 1.5-fold difference in the absorbance reading between bovine serum albumin (BSA) and rat immunoglobulin (IgG) at concentrations of $50\mu\text{g}/\text{ml}$ ($10\mu\text{g}/\text{well}$). Up to a two-fold difference has actually been seen in the absorbance readings between BSA and rat IgG at this concentration. For this reason, it is recommended to choose a protein standard with dye-binding characteristics most similar to the protein being assayed.

Quantify™ protein assay system applications

compatibility with numerous reagents

The Quantify™ Reagent is compatible with many common laboratory reagents and buffers used during protein purification and analysis. [Table 1](#) lists the maximum concentration of some reagents that can be present in the well during the assay. Because the Quantify™ Assay is very sensitive, protein samples often need dilution in order to produce a protein concentration that falls within the linear range of the assay. Since the dilution of the protein sample also dilutes the other materials in the solution, the absolute concentration of a substance that can be tolerated by the assay is often much higher than the concentration listed in [Table 1](#). In particular, nonionic detergents are poorly tolerated in the assay. However, if the protein solution is diluted sufficiently, the detergent interference may not be sufficient to prevent the use of the assay.

Table 1. Compatibility of Various Laboratory Reagents with the Quantify™ Protein Assay System.

Do Not Interfere*		Affect Sensitivity**		Completely Interfere***	
0.1%	CHAPS	50mM	EDTA	0.05%	deoxycholate
1.0M	glucose	1.0M	guanidine HCl	0.05%	Nonidet™ P-40
0.1M	glycine	0.1%	Tween® 20	0.05%	Triton® X-100
0.05%	sodium citrate	1.0M	b-mercaptoethanol	0.05%	Triton® X-114
0.5M	NaCl	0.01%	Triton® X-100	0.02X	CCLR
0.5%	NaN ₃	0.01%	Triton® X-114	0.02X	RLB
0.1N	NaOH	0.01X	CCLR		
0.01%	SDS	0.01X	RLB		
10%	sucrose				
0.1M	Tris				
100mM	HEPES				
10mM	DTT				
10%	glycerol				
0.1%	n-octylglucoside				

* Concentrations of these substances greater than those listed may interfere; concentrations less than those listed do not interfere.

** At the indicated concentrations, the presence of these substances will raise the background absorbance, limiting the detection sensitivity to $1\mu\text{g}$ protein per well ($5\mu\text{g}/\text{ml}$). Detection sensitivity in the presence of 0.01X CCLR or RLB cell lysis buffer is $2\mu\text{g}$ per well (equivalent to $1\text{mg}/\text{ml}$ in the undiluted cell lysate).

*** The presence of these substances at or above the indicated concentrations will cause background absorbance sufficient to completely interfere with the assay.

Abbreviations: CHAPS: (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate; SDS: sodium dodecyl sulfate; EDTA: ethylene diamine tetraacetic acid; HEPES: (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]); DTT: dithiothreitol (Cleland's Reagent).

Two reagents that normally interfere with the Bradford assay are Promega's Cell Culture Lysis Reagent (CCLR, Cat.# E1531) and Reporter Lysis Buffer (RLB, Cat.# E3971), which are used to lyse mammalian cells after transfection with reporter vectors. It is often useful to assay for total protein in these cell lysates in order to normalize the amount of expressed reporter enzyme to the total amount of protein in the lysate. However, the CCLR contains 1% Triton® X-100 and 10% glycerol, and the RLB contains Tween® detergents, all of which will interfere with the Bradford assay. Normal use of the CCLR and RLB results in mammalian lysates containing 1-2 mg protein per ml. These CCLR and RLB lysates must be diluted 100-fold in order to fall within the linear range of the Quantify™ Assay, effectively diluting the interfering substances. Thus, the assay is compatible with cell lysates made with CCLR and RLB reagents even though direct application of these reagents to the assay would not produce an acceptable background value.

Determination of relative protein concentrations in numerous samples and inclusion bodies

While the Quantify™ Assay was developed for the determination of the absolute concentration of protein in solutions, in some instances, the assay plates also can be used to determine relative concentration of protein in a number of samples. The plates are particularly useful for monitoring protein elution profiles during column chromatography. Many column fractions can be assayed in as little as 10 minutes and protein peaks are easily identified by their dramatic color change.

The Quantify™ Assay Reagent also can be used to determine the relative protein concentration present in inclusion bodies. Many recombinant proteins are produced as insoluble protein granules in prokaryotic host organisms. The protein granules, or inclusion bodies, are easily purified to near homogeneity by centrifugation of cell lysates of the production strains. However, most protein quantitation techniques cannot correctly determine the protein concentration of a purified inclusion body suspension. Since refolding studies often require the relative amount of protein to be known, researchers have resorted to estimating the protein concentration on SDS-polyacrylamide gels. The Quantify™ Reagent completely solubilizes inclusion body suspensions, allowing the protein concentration in the suspension to be rapidly and accurately determined.

Summary

The Quantify™ Protein Assay System rapidly determines the concentration of protein in solution. The reagent is supplied in a lyophilized form in a 96 well plate and is re-dissolved by the sample protein solution, effectively lowering the detection limit of the assay compared to liquid protein assay reagents. The system gives a linear response for protein amounts in the well from 200ng to 10µg, which corresponds to protein concentrations of 1-50µg/ml. The detection sensitivity of the Quantify™ Protein Assay System allows it to be used with some solutions which are difficult to analyze otherwise, such as cell lysates in Culture Lysis Reagent or Reporter Lysis Buffer and very dilute protein solutions.

References

1. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248

Ordering Information

Product	Size	Cat. #
Quantify™ Protein Assay System	4 96 Well Plates	V2650

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