Revised: 11–October–2001

CBQCA Protein Quantitation Kit (C-6667)

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Abs/Em: 465/550 nm

Number of Assays: 300–800 microplate assays

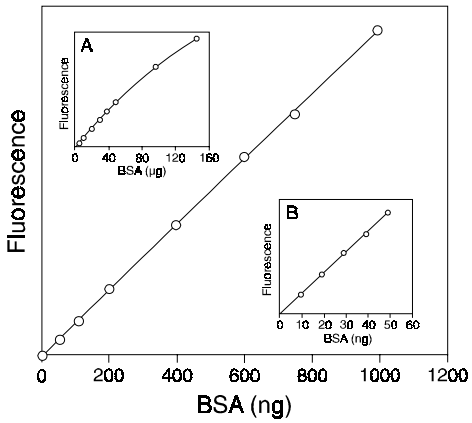

Figure 1. *Detection of bovine serum albumin (BSA) using the CBQCA Protein Quantitation Kit. The primary plot shows detection of BSA from 50 ng to 1000 ng. Inset A shows that the detection range can extend up to 150 µg. Inset B shows that the lower detection limit can extend down to 10 ng. For the assays in the low range, silanized glassware was used when preparing the BSA dilutions. Reactions were carried out in 100 µL volumes, and fluorescence was measured using a microplate reader with excitation at 485 ± 10 nm and emission detection at 530 ± 12.5 nm. All measurements were corrected for fluorescence of a control reaction lacking BSA. Each point is the average of four determinations.*

Introduction

Molecular Probes' CBQCA Protein Quantitation Kit provides a rapid and highly sensitive method for the quantitation of proteins in solution.[1] The kit utilizes the ATTO-TAG™ CBQCA reagent (3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde) originally developed as a chromatographic derivatization reagent for amines.[2] This reagent has also proven extremely useful for quantitating amines in solution, including the accessible amines in proteins. The ATTO-TAG CBQCA reagent is virtually nonfluorescent in aqueous solution; however, in the presence of cyanide, it reacts with primary amines such as those found in proteins to form highly fluorescent derivatives.

The CBQCA Protein Quantitation Kit supplies both the ATTO-TAG CBQCA and KCN required for this novel protein assay. To perform this simple assay, the protein samples are diluted into a suitable buffer, KCN is added and then the reaction is started by the addition of CBQCA from a freshly prepared working solution. After incubating for 1 hour (or up to 5 hours, if more convenient), the samples are read in a fluorescence microplate reader, standard fluorometer or minifluorometer using excitation/emission wavelength of approximately 465/550 nm. Bovine serum albumin (BSA) is provided with the kit for use as a protein standard. As little as 10 ng of BSA can be detected in a 100–200 µL assay volume using a fluorescence microplate reader, and the effective range for this assay extends up to 150 µg. Alternatively, the reaction mixtures can be diluted to 1–2 mL for fluorescence measurement in a standard fluorometer or minifluorometer. Standard curves for BSA samples ranging from 10 ng to 150 µg are plotted in Figure 1. The CBQCA protein quantitation assay also functions well in the presence of substances, such as lipids and detergents, which interfere with many other protein determination methods.[1] For example, the CBQCA-based assay can be utilized directly to determine the protein content of lipoprotein samples or lipid-protein mixtures (Figure 2).

Materials

Kit Contents

- **ATTO-TAG CBQCA derivatization reagent** (MW = 305, Component A), 5 mg
- **Dimethylsulfoxide (DMSO)** (Component B), 1 mL
- **Potassium cyanide** (MW = 65, Component C), ~22 mg
- **Bovine serum albumin (BSA) standard** (Component D), 4 mg

The CBQCA Protein Quantitation Kit supplies sufficient material for performing 300–800 assays, depending on the concentration of substrate used. Fluorescence of the 100–200 µL reactions may be measured either in a fluorescence microplate reader or in a standard fluorometer or minifluorometer by diluting the reaction mixture to 1–2 mL.

Storage and Handling

Upon receipt, this kit should be stored at -20°C protected from light. Solutions of Components A, C and D (prepared in steps 1.1–1.3) may be stored at 4°C for several days or at -20°C for long-term storage. The ATTO-TAG CBQCA stock solution in DMSO (and the DMSO from which it is made) will freeze at 4°C or below; allow the vial to warm to room temperature and mix well before opening. The ATTO-TAG CBQCA reagent should be protected from light. When properly stored, the components of this kit are stable for at least six months.

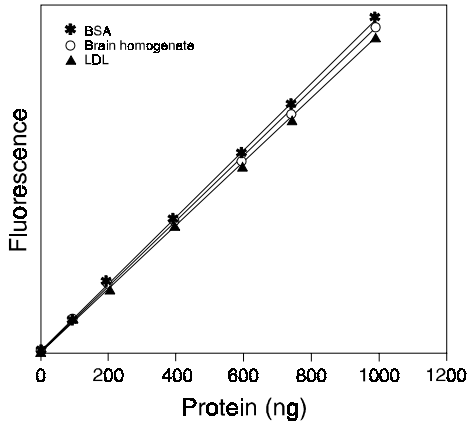

Figure 2. *Application of the CBQCA-based assay for the quantitation of lipoprotein and for the quantitation of protein in a lipid–protein mixture. The protein concentrations of an LDL preparation and a bovine brain homogenate were first determined by the modified Lowry method,[3] using BSA as a standard. Assays were then performed using the CBQCA Protein Quantitation Kit on samples containing from 100 ng to 1000 ng protein in 0.1 M sodium borate buffer, pH 9.3, containing 0.1% Triton® X-100. Similar results were obtained without the addition of detergent (data not shown). Fluorescence was measured as described for Figure 1 and corrected for fluorescence of a control reaction lacking protein. Each point is the average of three determinations.*

Materials Required But Not Provided

Reaction buffer is required but not provided. The protocol described below uses 0.1 M sodium borate, pH 9.3.

To prepare 100 mL of this buffer, dissolve 3.8 g of $Na_2B_4O_7 \bullet 10\ H_2O$ (MW 381) in about 80 mL of distilled water, adjust the pH to 9.3 if necessary and then add distilled water to 100 mL.

Experimental Protocol

Reagent Preparation

1.1 Prepare a 40 mM ATTO-TAG CBQCA stock solution by adding 410 µL of DMSO (Component B) to the ATTO-TAG CBQCA vial (Component A).

1.2 Prepare a ~20 mM KCN solution by dissolving the contents of the KCN bottle (Component C) in 17 mL of distilled water. **Warning:** *Potassium cyanide is highly toxic and should be handled with appropriate caution.*

1.3 Prepare a 4 mg/mL stock solution of BSA by dissolving the contents of the BSA vial (Component D) in 1.0 mL of distilled water or 0.1 M sodium borate, pH 9.3. We recommend that you add sodium azide to a final concentration of 2 mM as a preservative. The concentration of BSA can be verified by absorbance measurement; at 280 nm a 1 mg/mL solution of BSA has an absorbance of 0.60 in a cuvette with a 1 cm pathlength.

Protein Quantitation

The following protocol describes a typical reaction for the CBQCA Protein Quantitation Kit. In this example, the reaction volume is 150 µL, and the reactions can be carried out in a 96-well microplate for analysis in a fluorescence microplate reader. If desired, the reaction volume can be adjusted to 100–200 µL by changing the amount of buffer and/or protein sample added. For fluorometers that require a 1 or 2 mL volume, the reactions can be diluted after the incubation period.

The working solution of the ATTO-TAG CBQCA reagent (prepared in step 2.3) can be either 5 mM (for greatest sensitivity) or 2 mM (to maximize the number of assays). Approximately 300 assays can be performed with the 5 mM working solution, and approximately 800 assays with the 2 mM working solution. For assays of very small amounts of protein (10–100 ng), silanized glassware and pipets should be used.

2.1 Prepare the protein samples in 0.1 M sodium borate buffer, pH 9.3, to achieve a final volume of 135 µL. Always include a control with no protein added. In the presence of KCN, the ATTO-TAG CBQCA reagent reacts with the protein's accessible primary amines. The protein sample should therefore be free of ammonium salts and of contaminating amines such as Tris or glycine. In addition, thiols such as dithiothreitol or 2-mercaptoethanol at >100 µm final concentration should be avoided, as they will react with and inactivate the CBQCA reagent. Limited amounts of these contaminants can be tolerated if suitable controls are used.

2.2 Add 5 µL of 20 mM KCN (prepared in step 1.2) to each protein sample and mix well.

2.3 Allow the 40 mM ATTO-TAG CBQCA DMSO stock solution (from step 1.1) to thaw at room temperature. Prepare a 5 mM working solution (or 2 mM, see above) by diluting the stock solution in 0.1 M sodium borate buffer, pH 9.3. Each determination, including a blank and samples for a standard curve, will require 10 µL of this working solution; prepare a slight excess. *This aqueous working solution of CBQCA should be used immediately.*

2.4 Add 10 µL of the aqueous working solution of CBQCA to each KCN-protein sample and mix well.

Table 1. *Reaction mixtures for generating a BSA standard curve.*

Reaction Components	Amount of BSA in Reaction						
	0 ng	**50 ng**	**100 ng**	**200 ng**	**400 ng**	**700 ng**	**1000 ng**
Reaction buffer	135 µL	130 µL	125 µL	115 µL	95 µL	65 µL	35 µL
BSA (10 µg/mL)	0 µL	5 µL	10 µL	20 µL	40 µL	70 µL	100 µL
KCN	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL
CBQCA	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL

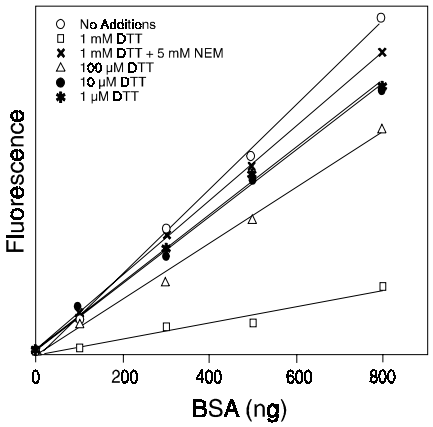

Figure 3. *Effect of thiols on protein determination in the CBQCA protein quantitation reaction. BSA samples ranging from 100 ng to 800 ng were assayed in 0.1 M sodium borate buffer, pH 9.3, alone or in the presence of the indicated concentrations of DTT or DTT plus NEM. Fluorescence was measured as described for Figure 1 and corrected for fluorescence of a no-BSA control reaction of the same composition. Each point is the average of three determinations.*

2.5 Protect the reactions from light, for example, by covering with aluminum foil. Incubate at room temperature with shaking for at least 1 hour.

2.6 After the incubation period, measure the fluorescence emission at ~550 nm with excitation at ~465 nm. Fluorescence may be read directly in a fluorescence microplate reader. Alternatively, the sample may be diluted in buffer to increase the volume and read in a standard fluorometer or minifluorometer. It is not practical, however, to perform the assay by adding the KCN and CBQCA working solutions directly to 1–2 mL of prediluted protein solutions. Set the plate reader or fluorometer for sensitivity appropriate to obtain sufficient fluorescence signal within the full measurement range. Subtract the fluorescence of the no-protein control from the fluorescence determined for each protein sample. The incubation period for the reaction does not need to be exactly 1 hour – if desired, the reactions can be incubated for up to 5 hours; however, the incubation period should be approximately the same for all samples, including standards and controls.

Protein Standard Curve

A reference standard curve can be generated for converting fluorescence observed in the assay to mass of protein in the sample. Ideally, the protein used for the standard curve should match the type of protein present in the experimental samples; however, as with other protein assays, BSA serves as a convenient reference standard.

3.1 Prepare a 10 µg/mL BSA solution by diluting 10 µL of the 4 mg/mL stock solution (from step 1.3) into 4.0 mL of reaction buffer (0.1 M sodium borate, pH 9.3, or other buffer to match the reaction conditions used for the experimental samples). If a standard curve with protein amounts greater than 1.5 µg is desired, a higher concentration BSA solution (e.g., 100 µg/mL) will also be required.

3.2 Prepare reactions containing variable amounts of the BSA standard. One possible set of reaction mixtures for generating a standard curve is presented in Table 1.

3.3 Mix and incubate the reactions, determine the fluorescence and correct for background fluorescence as described in *Protein Quantitation*.

3.4 Plot the corrected fluorescence versus mass of protein in the assay. An example of a more extensive standard curve is presented in Figure 1.

Tolerance for Additives in the Reaction

The CBQCA-based assay for protein quantitation can be performed under a wide range of reaction conditions; however, as mentioned above, amines (e.g., Tris or glycine) and ammonium ions should be avoided. The composition for no-protein control reactions and for standard curve reactions should always closely match that of the experimental samples. Figure 3 presents data confirming the assay's compatibility with concentrations of dithiothreitol (DTT) at ≤ 100 µM and shows that the interference caused by 1 mM DTT can be blocked by the inclusion of 5 mM *N*-ethylmaleimide (NEM). Similar results have been obtained in experiments with 2-mercaptoethanol (data not shown). Figure 4 presents data confirming the assay's compatibility with: 0.1% Triton® X-100, 0.1% Tween® 20, 0.1% sodium dodecyl sulfate (SDS), 10% glycerol, 10% sucrose, 30% CsCl or 0.1% (15 mM) sodium azide. Triton X-100, at a concentration of 0.1%, improves the sensitivity of the assay and can be added routinely, if desired.

Warning

Potassium cyanide is hazardous. Handle with extreme care. In particular, avoid exposing potassium cyanide to acid, which may cause the release of highly toxic hydrogen cyanide. Dispose of unused material in a safe and appropriate manner.

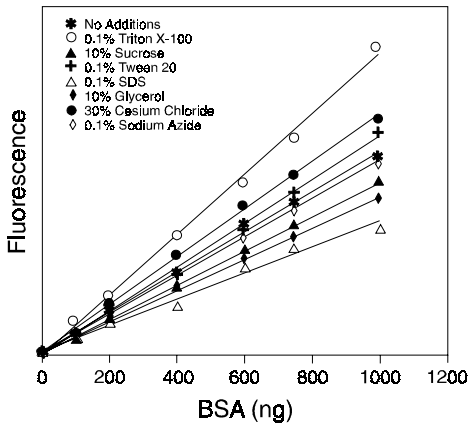

Figure 4. *Tolerance for additives in the CBQCA protein quantitation reaction. BSA samples ranging from 100 ng to 1000 ng were assayed in 0.1 M sodium borate buffer, pH 9.3, alone or in the presence of the indicated additives. Fluorescence was measured as described in Figure 1 and corrected for fluorescence of a no-BSA control reaction of the same composition. Each point is the average of four determinations.*

References

1. Anal Biochem 244, 277 (1997); **2.** Anal Chem 63, 408 (1991); **3.** Anal Biochem 83, 346 (1977).

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

Cat #	Product Name	Unit Size
C-6667	CBQCA Protein Quantitation Kit *300-800 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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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