# CBQCA Plus Protein Quantitation Kit

Catalog Number A66522

Pub. No. MAN1000130 Rev. A

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

The Invitrogen™ CBQCA Plus Protein Quantitation Kit provides a rapid and highly sensitive method for the quantitation of proteins in solution. The kit utilizes the ATTO-TAG™ CBQCA reagent (3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde), originally developed as a chromatographic derivatization reagent for amines. This reagent has also proven extremely useful for quantitating amines in solution, including the accessible amines in proteins. The ATTO-TAG™ CBQCA reagent is nonfluorescent in aqueous solution, and forms highly fluorescent derivatives in the presence of cyano groups on reacting with primary amines present in proteins.

The CBQCA Plus Protein Quantitation Kit is an updated version of the CBQCA Protein Quantitation Kit (Catalog No. C6667). It is designed to assist users in reducing or eliminating the use of potassium cyanide (KCN) in their laboratory experiments. This is achieved by replacing the KCN component with mandelonitrile, an alternative nitrile (CN) compound prepared in dimethyl sulfoxide (DMSO). Mandelonitrile exhibits similar reaction efficiency to KCN, making it an effective and less hazardous alternative.

## Contents and storage

| Item | Amount [1] | Storage and stability[2] |
|---|---|---|
| ATTO-TAG™ CBQCA derivatization reagent (MW = 305, Component A) | 5 mg | Store at –20°C |
| Dimethylsulfoxide (DMSO) (Component B) [3] | 1 mL | |
| 20 mM Mandelonitrile in DMSO (MW = 133.15, Component C) [4] | 4 mL | |
| Bovine serum albumin (BSA) standard (Component D) | 4 mg | |
| BupH™ Borate Buffer Pack | 1 EA | Store at room temperature |

[1] This kit supplies sufficient material for performing 300–800 assays depending on concentration of derivatization reagent used.
[2] Protect from light. The stability of these reagents lasts for 6 months after reconstitution, when stored properly.
[3] DMSO (Component B) freezes at 19°C.
[4] Mandelonitrile in DMSO (Component C) freezes at 19°C.

**Note:** Fluorescence of the 100–200 µL reactions can be measured in a fluorescence microplate reader. Alternatively, fluorescence can be read in a standard fluorometer or minifluorometer after diluting the reaction mixture to 1–2 mL.

## Procedural guidelines

- Mandelonitrile is more sensitive to pH changes than potassium cyanide (KCN).
- It is recommended to prepare a borate buffer at pH 8.5. This pH works well in most applications, but the assay can be performed at any pH between 8.5–9.3.

  **Note:** Performing the assay outside of this pH range will negatively impact product performance.
- BSA protein standard exhibits equivalent results between the two versions of the assay as depicted in Figure 1.
- For best results, users should use 2 mM CBQCA working reagent if the protein sample is a complex mixture such as a cell or tissue lysate.
- In protein quantitation, some level of protein-to-protein variability is expected due to the unique characteristics of each individual protein.

## Overview of procedure

### Prepare assay using CBQCA Plus Protein Quantitation Kit

Prepare assay using Bovine serum albumin (BSA) as a protein standard. Assay is capable of detecting around 50 ng of BSA in a 200 µL assay volume.

1. To perform the assay, dilute the protein samples with the included borate buffer, add mandelonitrile, and then initiate the reaction by adding CBQCA from a freshly prepared working solution.

2. After an incubation period of 1 hour (or a maximum of approximately 5 hours), read the results using a fluorescence microplate reader, standard fluorometer, or minifluorometer with an excitation/emission wavelength of approximately 465/550 nm.

   **Note:**
   - A reference standard curve can be generated for converting fluorescence observed in the assay to protein mass in the sample.
   - To reduce variability, the protein used for the standard curve should match the type of protein present in the experimental samples. As with other protein assays, Bovine serum albumin (BSA) serves as a convenient reference standard.

### Tolerance for additives in the reaction

- Primary amines (e.g., Tris or glycine) and ammonium ions must be avoided.
- The composition for no-protein control reactions and for standard curve reactions should always closely match that of the working solutions.
- Interference caused by reducing agents such as dithiothreitol (DTT) and β-mercaptoethanol at ≥100 µM can be blocked by the inclusion of 5 mM *N*-ethylmaleimide (NEM).
- Assay sensitivity can be increased by adding Triton X-100 to a final concentration of 0.1% v/v to both the samples and the BSA standards.
- Prepare or dilute samples in the supplied borate buffer for optimal performance.

**Note:** For more information about eliminating additives or interfering substances, go to **https://www.thermofisher.com**

### Prepare ATTO-TAG™ CBQCA stock solution

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).

**Table 1 ATTO-TAG™ CBQCA working solution for different assays**

| ATTO-TAG™ CBQCA stock solution | |
|---|---|
| **No. of assays** | **Amount** |
| 300 assays | 5 mM of stock[1] |
| 800 assays | 2 mM of stock[2] |

[1] For higher sensitivity
[2] To maximize the number of assays

**Note:** For assays which involve small amounts of protein (10–100 ng), low protein-binding plastic tubes and pipette tips must be used. As stated above, 2 mM working solution is recommended when assaying complex protein mixtures.

### Prepare borate buffer

Prepare 0.05 M borate buffer, pH 8.5 by dissolving the contents of the included BupH™ Borate Buffer Pack into 500 mL of deionized $H_2O$.

**Note:**

- After completion, the pH level is 8.5. If desired, the pH level can be adjusted between 8.5–9.3 by adding an appropriate concentration of sodium hydroxide.
- Using a pH value outside of this range is not recommended.

### Prepare BSA stock solution

Prepare a 4 mg/mL stock solution of BSA by dissolving the contents of the BSA vial (Component D) in 1.0 mL of 0.05 M borate buffer at pH 8.5.

**Note:**

- We recommend adding sodium azide as a preservative to a final concentration of 2 mM.
- Store at –20°C to 4°C.

## Prepare BSA reagent

Prepare a 10 µg/mL BSA solution by diluting 10 µL of the 4 mg/mL of BSA stock solution (Described in Prepare BSA stock solution) into 4.0 mL of 0.05 M borate buffer, pH 8.5.

**Note:**

- To achieve maximum sensitivity, low protein-binding tubes and pipette tips may be required.
- If a standard curve with protein amounts greater than 1.5 µg is desired, a higher concentration BSA solution (for example, 100 µg/mL) is required.
- The amount of BSA in the standard curve can range from 50 ng to 150 µg.
- Adjust the stock concentration of the BSA appropriately, using Table 2 as a reference.

## Protein quantitation procedure

1. Initiate reaction with CBQCA Plus Protein Quantitation Kit with a 150 µL total reaction volume.

   **Note:** Reactions can be carried out in a 96-well microplate for analysis in a fluorescence microplate reader. For fluorometers that require a 1 mL or 2 mL volume, the reactions can be diluted after the incubation period.

2. Prepare the protein samples in 0.05 M borate buffer, pH 8.5, to obtain a final volume of 135 µL.

3. Add 5 µL of 20 mM mandelonitrile (Component C) to each protein sample, followed by CBQCA to initiate the reaction. Do not combine mandelonitrile and CBQCA as a working reagent. Components must be added in sequential order as shown in Table 2.

4. Allow the ATTO-TAG™ CBQCA stock solution to thaw at room temperature. Prepare a 5 mM or 2 mM working solution (See Table 1) by diluting the stock solution in 0.05 M borate buffer, pH 8.5.

   **Note:**
   - This aqueous working solution of CBQCA must be used immediately.
   - Each determination, including a blank and samples for a standard curve, requires 10 µL of this working solution; prepare a slight excess.

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

**Table 2 Reaction mixtures for generating a BSA standard curve for samples containing 50–1000 ng BSA**

| Reaction components | Amount of BSA in reaction | | | | | | |
|---|---|---|---|---|---|---|---|
| BSA amount | 0 | 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 | 5 µL | 10 µL | 20 µL | 40 µL | 70 µL | 100 µL |
| Mandelonitrile | 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 |
| **Total reaction volume** | **150 µL** | **150 µL** | **150 µL** | **150 µL** | **150 µL** | **150 µL** | **150 µL** |

6. Incubate the reactions at room temperature with shaking for at least 1 hour, protected from light.

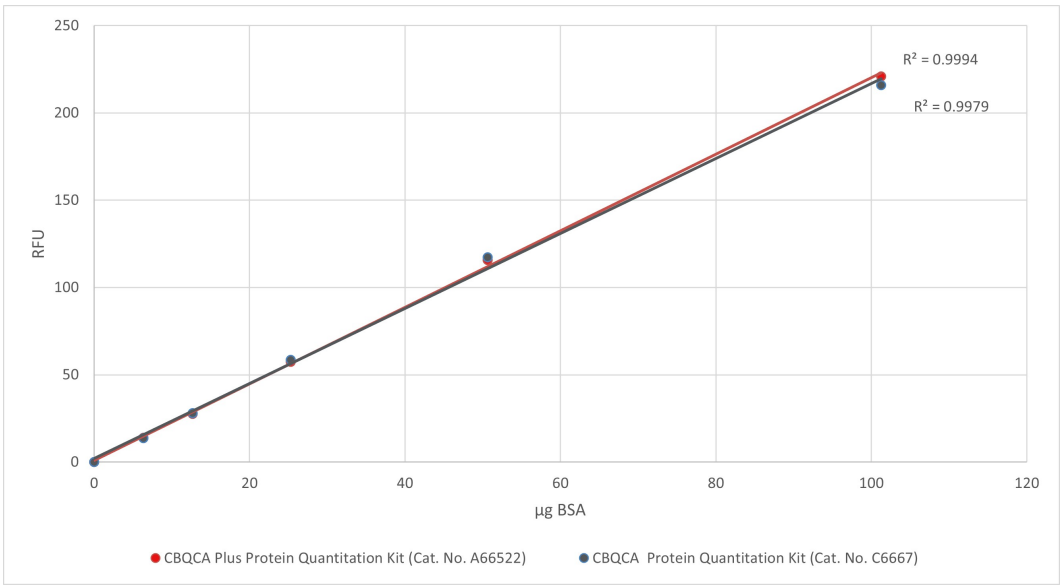
   **Note:**
   - The reactions can be incubated for up to 5 hours; however, the incubation should be the same for all samples, including standards and control.
   - Protect the reactions from light, by covering with aluminum foil.

7. After the incubation period, measure fluorescence emission at approximately 550 nm with an excitation at approximately 465 nm. Fluorescence can be directly read in a fluorescence microplate reader.

   **Note:** Alternatively, dilute the sample in a buffer to increase the volume and read in a standard fluorometer or minifluorometer.

8. Subtract the fluorescence of the no-protein control from the fluorescence determined for each protein sample. To generate a standard curve, plot the corrected fluorescence versus protein mass in the assay. An example of a standard curve is presented in Figure 1.

## Plot of fluorescence intensity vs protein mass in the assay



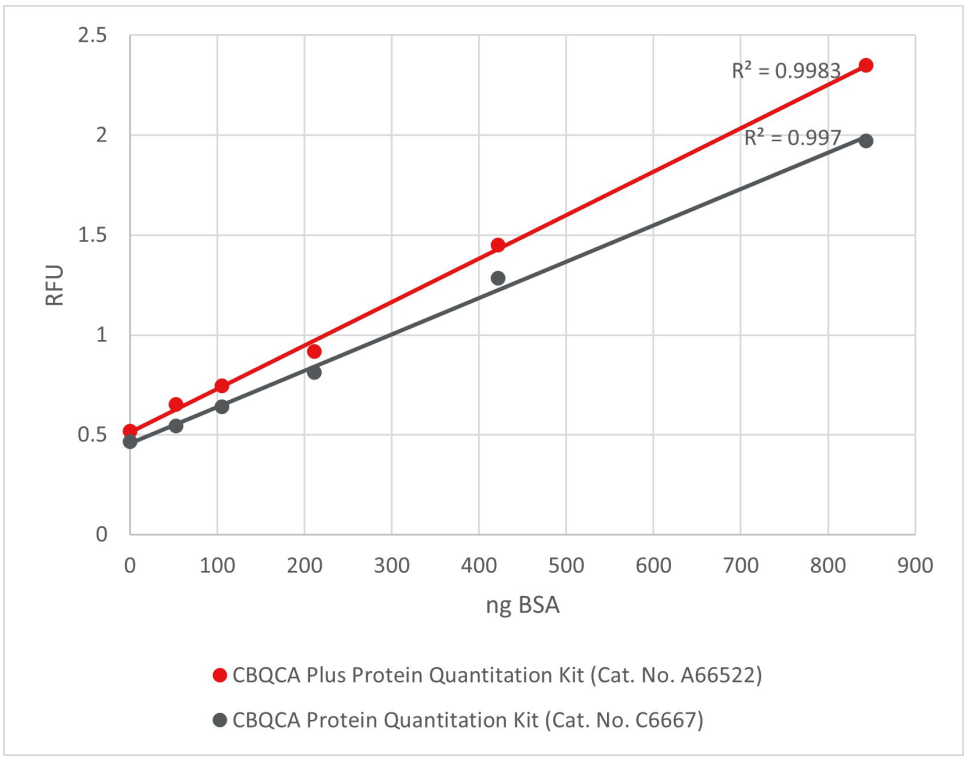




**Figure 1 Comparison of CBQCA Plus Protein Quantitation Kit (Cat. No. A66522) and CBQCA Protein Quantitation Kit (Cat. No. C6667)** Reactions were carried out in 150 µL volumes, and fluorescence was measured using a microplate reader with excitation at 465 ±12 nm and emission detection at 550 ±12 nm. (Top) BSA standard curve from 5 µg–100 µg. Fluorescence measurements were corrected by subtracting the fluorescence of a control reaction lacking BSA. (Bottom) BSA standard curve from 50 ng–1,000 ng.

## Related products

| Catalog No. | Product |
|---|---|
| 15041 | **Pierce™ 96-Well Polystyrene Plates, Corner Notch,** 100 plates |
| 15036 | **Sealing Tape for 96-Well Plates,** 100 sheets |
| 23209 | **Pierce™ Bovine Serum Albumin Standard Ampules,** 2 mg/mL |
| N6666 | **NanoOrange™Protein Quantitation Kit, 200–2,000 assays,** 1 kit |
| 23235 | **Pierce™MicroBCA Protein Assay Kit,** 500 mL |
| 23215 | **Compat-Able™Protein Assay Preparation Reagent Set,** 500 mL |
| A6222 | **ATTO-TAG™CBQCA Derivatization Reagent (CBQCA; 3-(4-Carboxybenzoyl)quinoline-2-Carboxaldehyde),** 10 mg |
| A10192 | **ATTO-TAG™ FQ Derivatization Reagent (FQ; 3-(2-Furoyl)quinoline-2-Carboxaldehyde),** 10 mg |
| A66521 | **ATTO-TAG™ FQ Plus Amine-Derivatization Kit** |

## Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

For troubleshooting information, to browse the database and search using keywords, visit **thermofisher.com/faqs**.

## Limited product warranty

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Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

**Revision history:** Pub. No. MAN1000130 A

| Revision | Date | Description |
|---|---|---|
| A | 13 May 2024 | Created new publication for CBQCA Plus Protein Quantitation Kit. |