Protein Determination Kit

Item No. 704002

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
704004	Protein Determination Assay Reagent	1 vial
704003	Protein Determination BSA Standard	1 vial
400010	96-Well Clear Plate	5 plates
400012	96-Well Cover Sheet	5 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 595 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

About This Assay

Cayman's Protein Determination Kit is a microplate based, colorimetric method for rapid total protein quantification. Based on the well-known Bradford method,[1,2] it takes advantage of the color change of Coomassie® Dye when it binds to proteins in acidic medium. When the dye binds, there is an immediate shift of the absorption maximum from 465 to 595 nm with a simultaneous change in color from brown to blue.

Performing the assay is simple: dilute your protein samples in water, pipette 100 µl of the diluted samples into a well of a 96-well plate, add 100 µl of assay reagent, incubate for five minutes and measure the absorbance at 595 nm. Protein concentrations are calculated from a regression line fit to a series of standard protein dilutions assayed on the same plate as the unknown samples.

PRE-ASSAY PREPARATION

Some kit components are in concentrated form and require dilution prior to use. Follow the directions carefully to ensure proper volumes of water are used to dilute the components. *NOTE: The quality of water used to dilute reagents and samples should be distilled or better. From this point forward, the use of the term "water" will imply "distilled or better."*

Reagent Preparation

1. **Protein Determination Assay Reagent - (Item No. 704004)**

   Dilute 7.5 ml of the Assay Reagent to 50 ml with water. This volume is enough for 200 samples in duplicate (40 per plate with a standard curve). Store in the dark (*e.g.*, in a brown bottle) at 4°C when not in use. The diluted reagent will be stable for one year, if stored properly.

2. **Protein Determination BSA Standard - (Item No. 704003)**

   The BSA standard is provided at a stock concentration of 10 mg/ml in a 0.9% saline solution with 0.05% sodium azide. Dilute 4 µl of the stock standard with 996 µl of water. The concentration of this solution (the bulk standard) will be 40 µg/ml.

   To prepare the standard for use in the assay: Obtain eight clean test tubes and label them A through H. Aliquot 200 µl of water to tube H and 250 µl to tubes A through G. Transfer 800 µl of the bulk standard (40 µg/ml) to tube H and mix thoroughly. Serially dilute the standard by transferring 750 µl from tube H to tube G; mix thoroughly. Next, transfer 750 µl from tube G to tube F; mix thoroughly. Repeat this process for tubes E through B. Tube A will serve as the blank for the standard curve. These diluted standards should not be used after more than 24 hours.

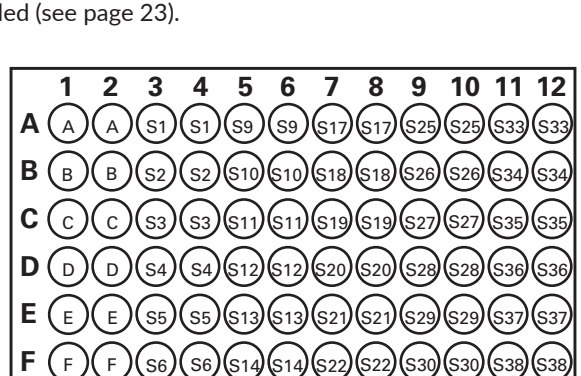
Standard	Final Concentration (µg/ml)
A	0.0
B	5.6
C	7.5
D	10.1
E	13.5
F	18.0
G	24.0
H	32.0

Table 1. Concentration of standards

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of standards and samples to be measured in duplicate is given below in Figure 1. The user may vary the location of wells as needed for the number of samples being assayed. We suggest you record the contents of each well on the template sheet provided (see page 23).



A-H = Standards
S1-S40 = Sample wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all wells.
- It is not necessary to use all the reagents at one time. However, a standard curve must be run simultaneously with each set of samples.
- If the concentration of protein in the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the standards and samples be assayed in triplicate.

Performing the Assay

Pipette the Reagents

1. **Protein Determination BSA Standard**

   Add 100 µl of standard (tubes A-H) per well in the designated wells on the plate (see Suggested Plate Format, Figure 1, page 8).

2. **Sample**

   Add 100 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed at least in duplicate (triplicate recommended). *NOTE: It has been our experience that samples resulting from the homogenization of tissues need to be diluted by at least a factor of 100 (i.e., 10 µl of sample + 990 µl of water).*

3. **Protein Determination Assay Reagent**

   Allow the Assay Reagent to warm to room temperature. Mix it immediately before use by gently inverting the container several times. Add 100 µl of Assay Reagent to each well being used.

Well	BSA Standard	Sample	Assay Reagent
Standard	100 µl	-	100 µl
Sample	-	100 µl	100 µl

Table 2. Pipetting summary

Incubation of the Plate

Incubate the plate for five minutes at room temperature.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Measure the absorbance at 595 nm. The dye color will be stable for at least one hour. After this time, dye-dye aggregates can form and are visible as a dark precipitate on the bottom of the wells.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. We recommend using a second order polynomial fit since the dye response at the standard concentrations is slightly non-linear. For a comparison of linear *versus* second-order polynomial curve fits see Figure 2 on page 13. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/) to obtain a free copy of this convenient data analysis tool.*

Calculations

If you must calculate the results manually, the procedure is as follows:

1. Average the absorbance of the blank wells (A1 & A2 in Table 1 on page 7), and subtract this value from all wells.
2. Average the pair of absorbance values for each standard.
3. Plot the average absorbance values at 595 nm of the standards as a function of BSA concentration and determine the best fit line through the points. An example of a typical standard curve is shown below. In general, because the concentration of the standards is low, a point-to-point line is a better approximation than a straight line.
4. Use the line from step 3 to estimate the concentrations of the samples from their average absorbance values.

Linear Regression (y = mx + b, where m = slope and b = y-intercept)

$$\text{Protein (µg/ml)} = \left[\frac{\text{sample absorbance - (y-intercept)}}{\text{slope}}\right] \times \text{Sample dilution}$$

Second-order Polynomial Fit ($y = Ax^2 + Bx + C$)

For ease of calculation, the plot should be constructed with concentration on the y-axis and absorbance on the x-axis.

$$\text{Protein (µg/ml)} = \left[A \times (\text{sample absorbance})^2 + B \times (\text{sample absorbance}) + C\right] \times \text{Sample dilution}$$

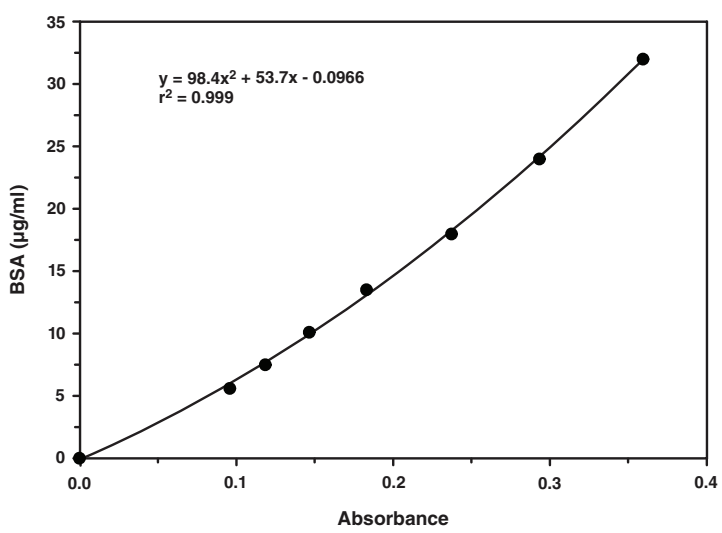


Figure 2. BSA standard curve with polynomial curve fit

Performance Characteristics

Sensitivity:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 5.6-32 µg/ml.

Response Characteristics of Various Proteins

All widely available total protein assay methods exhibit varying responses toward different proteins. The differences result from protein-to-protein variations in amino acid sequence, structure, presence of side chains, and pI, all of which can affect the protein's color response. The most common standards used in protein assay methods are BSA or immunoglobulin (IgG). The Cayman Protein Determination BSA Standard (Item No. 704003) is a consistent standard for the determination of protein concentration. Other proteins, however, vary in their color responses in the Cayman Protein Assay. Therefore, if great accuracy is required, the standard curve should be prepared from a pure sample of the protein being measured.

Table 3 (see page 15) shows the variation in color response of several proteins. All proteins were assayed, using the protocol described above, at a concentration of 14 µg/ml. The color response of BSA was normalized to 1.00 and the color response of the other proteins is expressed by the following ratio:

$$\frac{\text{Average Absorbance of "Test" Protein}}{\text{Average Absorbance of BSA}}$$

Figure 3 (see page 16) shows representative color response curves for BSA and insulin.

Protein	Ratio
BSA	1.00
Chymotrypsinogen	0.15
Cytochrome C	0.64
Myoglobin	1.16
IgG	0.36
Insulin	0.69
Transferrin	0.95

Table 3. Relative color response of various proteins

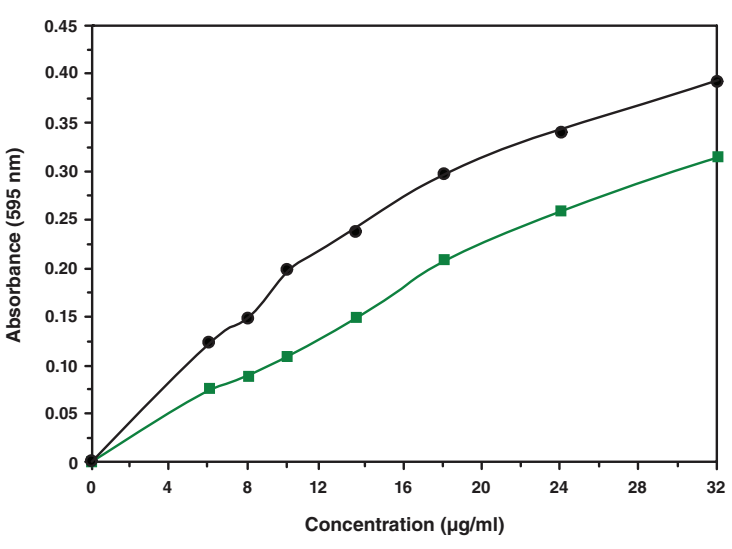


Figure 3. Representative response curves for BSA and insulin

Compatibility of Common Solutions and Solvents

Certain common laboratory solutions and solvents interfere with the Cayman Protein Determination Assay Reagent (Item No. 704004). For example, ionic and nonionic detergents can cause the Protein Determination Assay Reagent to precipitate and reduce color development. Other substances may interfere at their working concentrations (*e.g.*, 1.0 M ammonium sulfate), but cause no interference when the sample is diluted for use with the Protein Determination Assay Reagent. Since the assay uses BSA standards with relatively low concentrations, samples generally require some dilution to fall within the range of the standard curve. It is, however, a significant task to test for interference of all solutions that may be used in a laboratory environment. Generally a dilution of 1:50 eliminates potential interference from most common buffers and additives (see Table 4, on pages 18 and 19). In the event a 1:50 dilution cannot be used, Cayman recommends two methods to overcome any interference due to a particular solvent or solution:

1. Prepare the standard curve in the same solution your samples are in.
2. Spike an aliquot of the suspect solution and an aliquot of water with a known concentration of BSA. Compare the calculated concentrations of both solutions. If they are within 20%, the solution is considered compatible.

Solution	Concentration
Acetonitrile	10%
Ammonium sulfate	100 mM
Ascorbic acid	50 mM
Calcium chloride in TBS, pH 7.2	10 mM
Cobalt chloride in TBS, pH 7.2	10 mM
Cysteine	10 mM
Dithiothreitol (DTT)	5 mM
DMSO	10%
EDTA	100 mM
EGTA	2 mM
Ethanol	10%
Glycerol	10%
HEPES, pH 7.5	100 mM
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
MES (0.1 M), NaCl (0.9%), pH 4.7	
MES, pH 6.1	100 mM
Methanol	10%
MOPS, pH 7.2	100 mM
Nickel chloride in TBS, pH 7.2	10 ml

Table 4. Compatible solutions and solvents when diluted by a factor of 50 or greater

Solution cont.	Concentration cont.
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	
Sodium carbonate/sodium bicarbonate, pH 9.4	200 mM
Sodium citrate, pH 6.4	200 mM
Sodium phosphate	100 mM
Sodium thiocyanate	3.0 M
Sucrose	10%
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	
Triethanolamine, pH 7.8	100 mM
Tris (25 mM), Glycine (192 mM), pH 8.0	
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3	
Zinc chloride in TBS, pH 7.2	10 mM

Table 4. Compatible solutions and solvents when diluted by a factor of 50 or greater continued

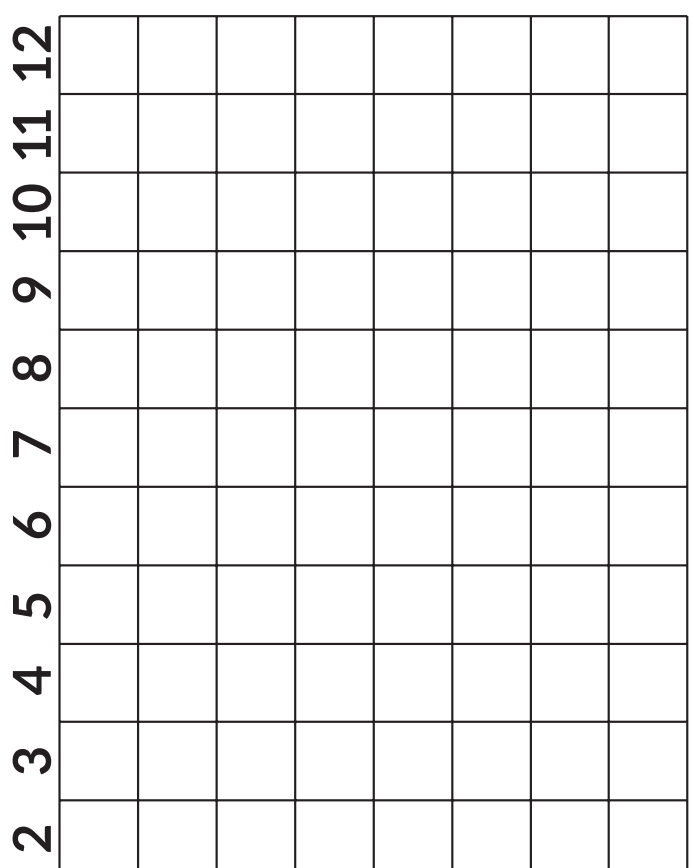
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Absorbance of standards and samples lower than expected	A. Reagent cold or stored improperly B. Absorbance not measured at 595 nm	A. Allow reagent to warm to room temperature B. Store reagents at 4°C C. Read absorbance at 595 nm
Calculated concentrations of samples lower than expected	Protein has molecular weight <3,000	Use a protein assay based on the BCA or Lowry method
Plate reader cannot read at 595 nm	Reader does not have 595 nm filter	A. Absorbance may be read at any wavelength from 575 nm to 615 nm B. The sensitivity of the assay, however, may be reduced
Precipitate forms in all wells	A. Sample contains a detergent B. Samples not mixed well or allowed to stand for several hours before reading	A. Dialyze or dilute samples to decrease or remove detergent B. Mix samples, by pipetting up-and-down several times, prior to reading
All sample wells are dark blue	Samples are too concentrated	Dilute samples

References

1. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254 (1976).
2. Sedmak, J.J. and Grossberg, S.E. A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. *Anal. Biochem.* **79**, 544-552 (1977).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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