CB™ Protein Assay

A Coomassie Dye Based Protein Assay
An Improved Bradford Assay

INTRODUCTION

An improved Coomassie Dye based protein assay based on the Bradford Protein Assay [1]. This assay is suitable for the simple and rapid estimation of protein concentration and detects proteins in the range of 1-1,000µg/ml. This assay is based on a single Coomassie dye based reagent. The binding of protein to the dye results in a change of color from brown to blue. and this change in color density is proportional to protein concentration. Protein estimation can be performed using as little as 0.5µg protein. The improved version greatly improves the linear range of the standard curve, a problem inherent with Coomassie based assays.

The protein-dye complexes reach a stable end point in 5 minutes. The CB™ Protein Assay is compatible with reducing agents and a wide variety of common laboratory agents listed below.

The CB™ Protein Assay has sufficient reagents for 500 standard test tube assays, 2,500 standard microwell assays, 1000 dilute test tube assays or 5,000 dilute microwell assays.

ITEM(S) SUPPLIED **786-012T** **Cat# 786-012**

Description	Size	Size
CB-Protein Assay Reagent	15ml	500ml
Bovine Serum Albumin (BSA) Standard (2mg/ml)	N/A	5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Store it at 4°C, upon arrival. When stored and used as recommended, the reagent is stable for one year.

MATERIAL NEEDED BUT NOT SUPPLIED

- Disposable 1ml polystyrene cuvettes (Cat. # 786-009), 2ml assay tubes (Cat. # 786-008).

PREPARATIONS BEFORE USE

1. Mix the CB™ Protein Assay Reagent by gently inverting the bottle, DO NOT SHAKE TO MIX.
2. Remove the appropriate amount of reagent required for the assay and allow to warm to room temperature.

PROTOCOLS

1. Preparation of Protein Standards
2. Microplate or Microwell (200µl) Assays:
   A. Standard Assay for protein concentrations of 100-1000µg/ml.
   B. Dilute Assay for protein concentrations of 1-25µg/ml.
3. Test Tube (1ml) Assays:
   A. Standard Assay for protein concentrations of 100-1000µg/ml.
   B. Dilute Assay for protein concentrations of 1-25µg/ml.

1. PREPARATION OF PROTEIN STANDARDS

For minimizing interference, it is important to prepare the appropriate diluted protein standard in the same diluent used for the test protein sample. For the Dilute Protocol, prepare a 0.1mg BSA/ml stock solution by mixing 50µl 2mg/ml stock with 950µl diluent. Use this stock for preparing diluted protein standard for the micro protocol assay.

FOR STANDARD PROTOCOL (25-2000µg/ml)

2mg/ml BSA STANDARD (µl)	*DILUENT* (µl)	*FINAL BSA CONCENTRATION (µg/ml)*
400	0	2000
300	100	1500
200	200	1000
150	250	750
100	300	500
50	350	250
25	375	125
5	395	25
0	400	0 (Blank)

FOR DILUTE PROTOCOL (2.5-25µg/ml)

0.1mg/ml BSA STANDARD (µl)	*DILUENT* (µl)	*FINAL BSA CONCENTRATION (µg/ml)*
250	750	25
200	800	20
150	850	15
100	900	10
50	950	5
25	975	2.5
0	1000	0 (Blank)

2A. STANDARD MICROPLATE OR MICROWELL ASSAY: (For protein concentrations of 100-1000µg/ml)

We recommend that the assays are performed in duplicate.

1. Transfer 10µl diluted standards, blank and test samples into microwells.
2. Gentle invert the CB™ Protein Assay reagent and add 200µl into each well and mix well. Incubate at room temperature for 5 minutes for optimal results. Do not exceed a 60 minute incubation.
3. Read optical density of the assay tubes at 595nm.
4. Subtract the average absorbance at 595nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.

2B. DILUTE MICROPLATE OR MICROWELL ASSAY: (For protein concentrations of 1-25µg/ml)

We recommend that the assays are performed in duplicate.

1. Transfer 100µl diluted standards, blank and test samples into microwells.
2. Gentle invert the CB™ Protein Assay reagent and add 100µl into each well and mix well. Incubate at room temperature for 5 minutes for optimal results. Do not exceed a 60 minute incubation.
3. Read optical density of the assay tube at 595nm.
4. Subtract the average absorbances at 595nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.

3A. STANDARD TEST TUBE (1ml) ASSAY: (For protein concentrations of 100-1000µg/ml)

We recommend that the assays are performed in duplicate.

1. Transfer 50µl diluted standards, blank and test samples into assay tubes or micro centrifuge tubes.
2. Gentle invert the CB™ Protein Assay reagent and add 1ml into each tube and mix well. . Incubate at room temperature for 5 minutes for optimal results. Do not exceed a 60 minute incubation.
3. Read optical density of the assay tubes at 595nm.
4. Subtract the average absorbances at 595nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.

3B. DILUTE TEST TUBE (1ml) ASSAY: (For protein concentrations of 1-25µg/ml)

We recommend that the assays are performed in duplicate.

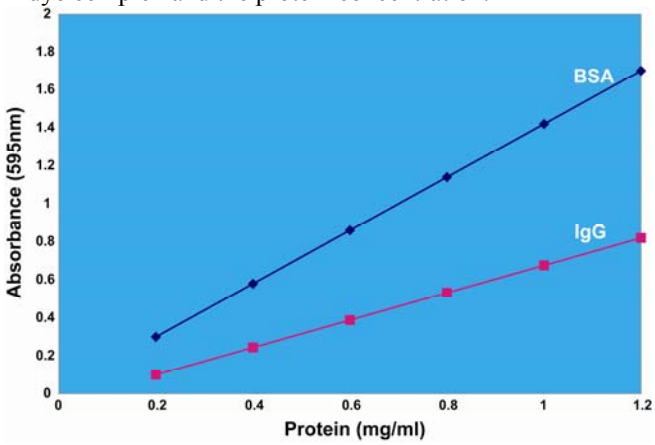
1. Transfer 0.5ml diluted standards, blank and test samples into assay tubes or micro centrifuge tubes.
2. Gentle invert the CB™ Protein Assay reagent and add 0.5ml into each tube and mix well. . Incubate at room temperature for 5 minutes for optimal results. Do not exceed a 60 minute incubation.
3. Read optical density of the assay tubes at 595nm.
4. Subtract the average absorbances at 595nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.

Standard Curve for the CB™ Protein Assay

If a curve-fitting algorithm is used when reading microwell plates on a plate reader, we recommend using a quadratic or best-fit curve for more accurate results. than a purely linear fit.

The 595nm absorbances may be lower with the Standard microwell assays compared to Standard test tube assays due to a shorter light path. If higher absorbances are required, we recommend using 15µl protein samples and 300µl CB™ Protein Assay reagent.

Within the recommended protein concentration range, the CB™ Protein Assay shows a substantially linear relationship between optical density of protein-dye complex and the protein concentration.



PROTEIN-TO-PROTEIN VARIATION

Protein-dye complex color is primarily the result of binding of the Coomassie dye to the basic and aromatic amino acid residues, especially arginine; therefore, the Coomassie dye based protein assays show protein-to-protein variations. Response of the *CB*-Protein Assay™ to albumin and γ-globulin is shown above. Protein concentration is generally measured using either BSA or γ-globulin as a protein standard. For greater accuracy, the standard plot should be prepared using a protein sample that has a color response similar to the test sample. Ideally, a pure fraction of the test protein.

INTERFERENCE TO PROTEIN ASSAY

The following table lists the agents compatible with the CB™ Protein Assay. The table also shows the acceptable concentration of reagents for standard protocols. In most cases, using a correct blank will eliminate or minimize the error caused by interference.

Compounds	Concentration	Compounds	Concentration	Compounds	Concentration
Amino acids	1mM	DTT	1M	Phenol	5%
Ammonium sulfate	1M	EDTA	100mM	Sodium azide	0.5%
Ampholytes	0.5%	EGTA	50mM	Sodium chloride	6M
Ascorbic acid	50mM	Ethanol	10%	Sodium dodecyl sulfate (SDS)	0.015%
Boric acid	1mM	Glucose	1M	Sodium hydroxide	0.1M
Brij® 35	0.06%	Glycerol	10%	Sodium phosphate	0.1M
CHAPS	0.5%	Glycine	0.1M	Sucrose	25%
CHAPSO	0.5%	Guanidine.HCl	6M	Tris	2M
Citrate	0.05%	HEPES	0.1M	Triton® X-100, X-114	0.06%
Cysteine	10mM	2-mercaptoethanol	1M	tRNA	0.35mg/ml
Deoxycholate	0.1%	Methanol	10%	Tween® 20	0.03%
DMSO	10%	MES	0.7M	Urea	3M
DNA	1mg/ml	Nonidet® P-40	0.5%		

TROUBLESHOOTING:

Protein solution contains interfering agents:
Remove interfering agents by dialysis or other methods. Alternatively, use a different protein assay:
Non-Interfering™ (NI™) Protein Assay (Cat. # 786-005): Unaffected by interfering assays.
CB-X™ Protein Assay (786-12X): Contains a protein clean up component.

Reagent Bottle Shows Precipitation:
Mix the reagent in the bottle gently by inverting the bottle several times. Do not shake the bottle.

Staining of Glassware:
Clean glassware with methanol.

Poor Color Development:
Reagents may be too old and expired.

Effect of Temperature:
Consistent results are obtained when CB™ Protein Assay is at room temperature. Allow CB™ Protein Assay to warm to room temperature.

RELATED PRODUCT
Non-Interfering Protein Assay™ *- A protein assay that overcomes interference of agents commonly present in protein solutions and shows no protein-to-protein variation. This assay has been extensively tested to work in the presence of common laboratory agents, such as reducing agents (2ME, DTT), chelating agents EDTA, detergents, Tris, urea, guanidine hydrochloride, and numerous other agents. The Non-Interfering Protein Assay™ is not affected even if your protein is in extraction buffers containing [4M urea, 1% SDS, 10mM EDTA, and 0.8% 2ME], [1% Sarcosyl, 4M guanidine thiocyanate, 10mM EDTA, and 0.5% 2ME] and other strong extraction buffers.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*