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A Geno Technology, Inc. (USA) brand name

CB™ Protein Assay

A Coomassie Dye Based Protein Assay; An Improved Bradford Assay

(Cat. # 786-012, 786-012T, 786-893)

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

Description	**Cat. # 786-012T**	**Cat. # 786-012**	**Cat. # 786-893**
CB™ Protein Assay Reagent	15ml	500ml	500ml
Bovine Serum Albumin (BSA) Standard [2mg/ml]	-	5ml	-
Non-Animal Protein Standard [2mg/ml]	-	-	5ml

STORAGE CONDITIONS

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.

ADDITIONAL ITEMS REQUIRED

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

PREPARATION 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.

PROTOCOL: 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/ml protein standard 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)

Bovine Serum Albumin or Non-Animal Protein Standard (µl)	Diluent (µl)	Final Standard 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 Bovine Serum Albumin or Non-Animal Protein Standard (µl)	Diluent (µl)	Final Standard 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)

PROTOCOL: 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.

PROTOCOL: 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.

PROTOCOL: 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.

PROTOCOL: 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.

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.

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

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. 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.

TROUBLESHOOTING

Protein solution contains interfering agents

Remove interfering agents by dialysis or other methods. Alternatively, use a different protein assay. We recommend Non-Interfering™ (NI™) Protein Assay (Cat. # 786-005) or CB-X™ Protein Assay (786-12X).

Reagent Bottle Shows Precipitation

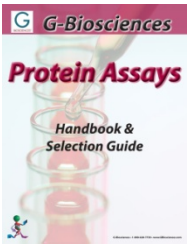
Mix the reagent in the bottle gently by inverting the bottle several times. Do not shake the bottle.

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 PRODUCTS

Download our Protein Assays Handbook.



http://info.gbiosciences.com/complete-protein-assay-guide

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