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A Geno Technology, Inc. (USA) brand name

# DCB™ Protein Assay

Detergent Compatible Coomassie Dye (Bradford)  
Assay

(Cat. # 786-1594T, 786-1594)



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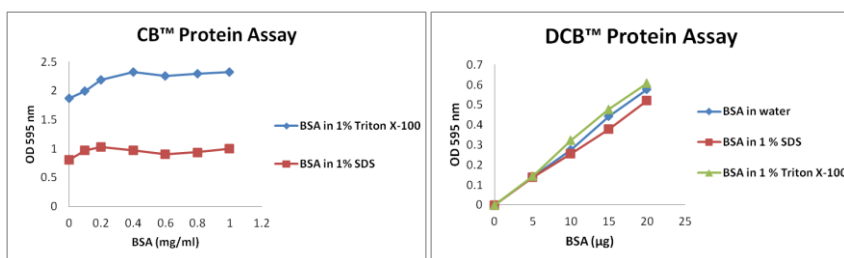
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## INTRODUCTION

DCB™ Protein Assay is a Coomassie Dye (Bradford) based detergent compatible assay. The Assay contains proprietary reagents suitable for samples containing detergents including SDS and Triton-X 100. DCB™ Protein Assay is simple and rapid to perform with reaction optimum time of 5 minutes. DCB™ Protein Assay can also be used for samples that do not contain detergents.

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 0.5 µg protein. The protein-dye complexes reach a stable endpoint in 5 minutes.

DCB™ Protein Assay has sufficient reagents for 500 test tube assay or 2,500 standard microwell assays.



**Fig.1:** BSA standard with and without detergents compared with CB™ Protein Assay (left) and DCB™ Protein Assay (Right).

## ITEM(S) SUPPLIED

Description	Cat. # 786-1594T	Cat. # 786-1594
DCB™ Protein Assay Reagent	15 ml	500ml
Bovine Serum Albumin (BSA) 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.

## IMPORTANT INFORMATION

- Bring the assay reagents to room temperature. Invert DCB™ Protein Assay Reagent bottle 2-3 times to mix before opening the reagent.
- DCB™ Protein Assay can be used with samples that contain or do not contain detergents.

- Assay incubation time is 5 minutes, although sample and standards can be measured anytime within 60 minutes of the reaction.

### ADDITIONAL ITEMS REQUIRED

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

### PROTOCOL

1. Mix the DCB™ 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 it to warm to room temperature.
3. Prepare BSA standard as below

**NOTE:** For minimizing interference, it is advised to prepare the appropriate diluted protein standard in the same diluent used for the test protein sample.

#### For Standard for test tube assay (125-1000µg/ml)

Bovine Serum Albumin (BSA) Standard [2mg/ml] (µl)	Diluent (µl)	Final Standard Concentration (µg/ml)
100	100	1000
75	125	750
50	150	500
25	175	250
12.5	187.5	125
0	200	0 (Blank)

**NOTE:** This is just an example. One can make their own standard as per requirement as the detection range of the assay is 1-1000 µg (Fig.1)

#### Protocol for Standard Microplate or Microwell Assay

We recommend that the assays are performed in duplicate.

1. Transfer 10µl diluted standards, blank and test samples into microwells.
2. Add 200 µl of DCB™ Protein Assay Reagent per well and mix well.
3. Incubate the plate at room temperature for 5 minutes.

**NOTE:** 5 minute incubation is for optimal results. Do not exceed more than 60 minutes incubation.

4. Read absorbance at 595nm on a microplate or ELISA plate reader.
5. 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 for Standard Test Tube (1 ml) Assay

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. Add 1ml of DCB™ Protein Assay reagent into each tube and mix well. Incubate at room temperature for 5 minutes.

**NOTE:** 5 minute incubation is for optimal results. Do not exceed more than 60 minutes incubation.

3. Read absorbance at 595nm using disposable 1ml polystyrene cuvettes.
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 DCB™ 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.

### 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.

### INTERFERENCE TO PROTEIN ASSAY

The following table lists detergents compatible with the DCB™ Protein Assay. The table also shows the acceptable concentration of detergents for standard protocols.

Detergents	Compatible concentrations
Brij™-35	1%
Brij-58	1 %
CHAPS , CHAPSO	5%
Nonidet P-40	1%
SDS	2%
Triton™ X-100, X-114	1 %
Tween™ 20	1%
Tween 80	0.1 %

**NOTE:** Detergents in samples were considered suitable for DCB™ Protein Assay if the error in protein concentration of BSA in the presence of detergents in sample is less than or equal to 10%.

## TROUBLESHOOTING

### ***Reagent Bottle Shows Precipitation***

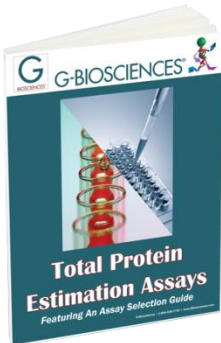
Mix the reagent in the bottle gently by inverting the bottle several times. Do not shake the bottle.

### ***Effect of Temperature***

Consistent results are obtained when DCB™ Protein Assay reagents are brought to room temperature. Allow DCB™ Protein Assay reagents to warm to room temperature.

## RELATED PRODUCTS

Download our Protein Assays Handbook.



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

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.





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