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A Geno Technology, Inc. (USA) brand name

RED 660™ Protein Assay

A Ready-To-Use Colorimetric Protein Assay

(Cat. # 786-676, 786-899)

INTRODUCTION

RED 660™ Protein Assay is a single reagent colorimetric assay that outperforms commercial colorimetric assays, including Bradford and improved Coomassie/ Bradford assays. RED 660™ Protein Assay offers greater linearity, greater color stability, and greater compatibility with detergents, reducing agents and other interfering agents compared to the Coomassie assays. The single, ready-to-use reagent allows for rapid analysis of total protein concentration and generates highly reproducible results.

This assay is suitable for the simple and rapid estimation of protein concentration and detects proteins in the range of 50-2000µg/ml. This assay is based on a single proprietary dye-metal complex reagent. The binding of protein to the dye-metal complex under acidic conditions results in a change of color from reddish-brown to green and this change in color density is proportional to protein concentration. The color change is a result of deprotonation of the dye-metal complex at low pH, which is facilitated by interactions with positively charged amino acid groups. Protein estimation can be performed using as little as 0.5µg protein. The protein-dye complexes reach a stable end point in 5 minutes, remaining stable for several days.

The RED 660™ Protein Assay has sufficient reagents for 500 standard test tube assays or 2,500 standard microwell assays.

ITEM(S) SUPPLIED (Cat. # 786-676)

Description	Cat. # 786-676	Cat. # 786-899
RED 660™ Protein Assay Reagent	2 x 250ml	2 x 250ml
Bovine Serum Albumin (BSA) Standard (2mg/ml)	5ml	-
Non-Animal Protein Standard (2mg/ml)	-	5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Store RED 660™ Protein Assay Reagent at room temperature and Protein Standard 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)
- Microplate
- Optional: Neutralizer™ (Cat. # 786-673) for samples with >0.01% SDS or in Laemmli buffer

PROTOCOLS

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 STANDARD PROTOCOL (25-2000µg/ml)

2mg/ml Protein Standard (µl)	Diluent (µl)	Final Protein Concentration (µg/ml)
400	0	2000
300	100	1500
200	200	1000
150	250	750
100	300	500
50	350	250
25	375	125
10	390	50
0	400	0 (Blank)

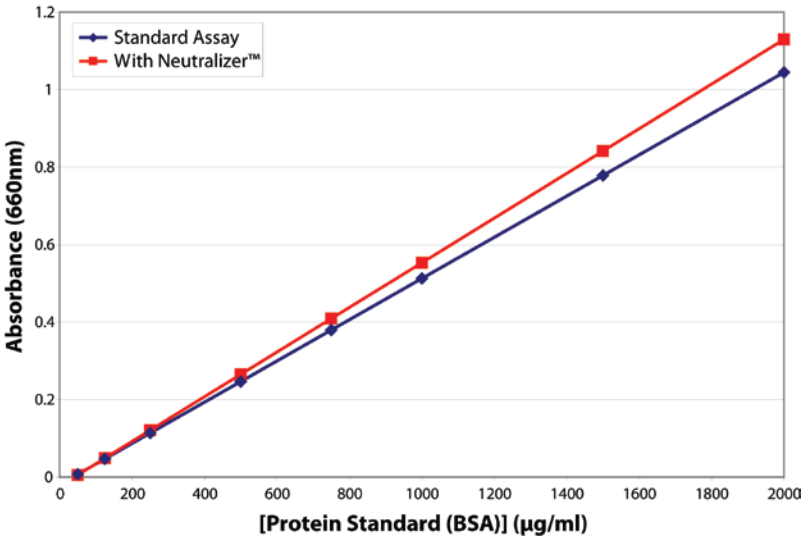


Figure 1: Standard Protein Curve in Absence and Presence of Neutralizer™

2. Sample Preparation

For samples with >0.01% SDS or other interfering ionic detergents

Determine the protein concentration with 10ml RED 660™ Protein Assay Reagent supplemented with one vial of Neutralizer™. Simply add and vortex until completely dissolved. This solution is stable for 1 day at room temperature.

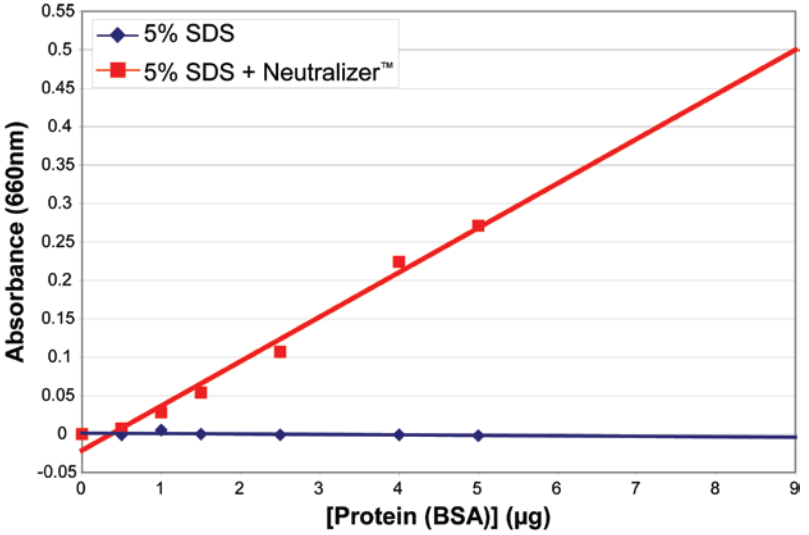


Figure 2: Presence of Neutralizer™ overcomes 5% SDS interference.

For samples in Laemmli loading buffer

Samples directly lysed in Laemmli buffer, should be diluted 1:10 to 1:20 in Laemmli buffer and the protein concentration determined with 10ml RED 660™ Protein Assay Reagent supplemented with one vial of Neutralizer™. Simply add and vortex until completely dissolved. This solution is stable for 1 day at room temperature.

For samples in RIPA Buffer (Cat. # 786-490)

For samples in RIPA buffer, add Triton® X-100 to a final concentration of 0.8%. For example for a 100µl assay sample, combine 92µl RIPA buffer lysed sample with 8µl 10% Triton® X-100. Perform assay as described and multiply the protein concentration by the dilution factor (1.087).

3. 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 200µl RED 660™ Protein Assay Reagent into each well and mix well by pipetting up and down.
3. Incubate at room temperature for 5 minutes for optimal results.
4. Vortex samples and then immediately read optical density of the assay tubes at 660nm.
   NOTE: *If a 660nm filter is unavailable, the assay can be read between 645-670nm, however this will result in a decrease in the linear range and also result in a decrease insensitivity.*
5. Subtract the average absorbances at 660nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.
   NOTE: *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.*

4. Standard Tube Assay

We recommend that the assays are performed in duplicate.

1. Transfer 50µl diluted standards, blank and test samples into suitable assay tubes.
   NOTE: *Smaller sample volumes can be used as long as a ratio of 1:20 Sample to RED 660™ Protein Assay Reagent is maintained.*
2. Add 1ml RED 660™ Protein Assay Reagent into each well and mix well.
3. Incubate at room temperature for 5 minutes for optimal results.
4. Vortex samples and then immediately read optical density of the assay tubes at 660nm.
   NOTE: *If a 660nm filter is unavailable, the assay can be read between 645-670nm, however this will result in a decrease in the linear range and also result in a decrease insensitivity.*
5. Subtract the average absorbances at 660nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.
   NOTE: *If a curve-fitting algorithm is used, we recommend using a quadratic or best-fit curve for more accurate result than a purely linear fit.*

INTERFERENCE TO PROTEIN ASSAY

The following table lists the agents compatible with the RED 660™ 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. * Indicates acceptable concentration when RED 660™ Protein Assay Reagent is supplemented with Neutralizer™.

Compounds		**Compounds**	
Acetone	50%	HCl	125mM
Acetonitrile	50%	Imidazole, pH7.0	200mM
Ammonium sulfate	125mM	Mammalian PELB™	Dilute 2-fold
Ascorbic acid	500mM	2-mercaptoethanol	1M
Bacterial PELB™	Dilute 2-fold	Methanol	50%
Borate buffer, pH8.5	50mM	MES, pH 6.1	125mM
Brij® 35	5%	MOPS, pH7.2	125mM
Carbonate-bicarbonate, pH9.4	Dilute 3-fold	Nonidet® P-40	5%
CHAPS	5%	Octylthioglucopyranoside	10%
CHAPSO	4%	Octyl-β-glucoside	5%
Citrate	12.5mM	Phenol red	0.5mg/ml
CTAB*	2.5%	PIPES, pH6.8	100mM
Cysteine	350mM	Sodium acetate, pH4.8	100mM
Deoxycholate	0.25%	Sodium chloride	1.25M
DMF	50%	SDS	0.0125%, 5%*
DMSO	50%	Sodium hydroxide	0.125M
DTT	500mM	Sucrose	50%
EDTA	20mM	TCEP	40mM
EGTA	20mM	Thiourea	2M
Ethanol	50%	Tissue PELB™	Dilute 2-fold
FOCUS™ Extraction Buffers	Compatible	Tris.HCl, pH8.0	250mM
Glutathione (Reduced)	100mM	Triton® X-100	1%
Glycerol	50%	Triton® X-114	0.5%
Glycine buffer. pH2.8	0.1M	Tween® 20	10%
Guanidine.HCl	2.5M	Urea	8M
HEPES, pH7.5	0.1M		

Table 1: Maximum compatible substances for RED 660™ Protein Assay

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 histidine, arginine and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine; therefore, the RED 660™ Protein Assay shows protein-to-protein variations (Table 2). 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.

Protein	Ratio	Protein	Ratio
Aldolase	0.83	Human Transferrin	0.8
Bovine Gamma Globulin	0.51	α-lactalbumin	0.82
Bovine Pancreas Insulin	0.81	Lysozyme	0.79
BSA (Bovine serum albumin)	1.00	Mouse IgG	0.48
Horse Heart Cytochrome C	1.22	Ovalbumin	0.54
Horse Heart Myoglobin	1.18	Rabbit IgG	0.38
Human IgG	0.57	Soybean Trypsin Inhibitor	0.38

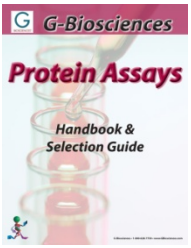
Table 2: Protein-to-Protein Variation

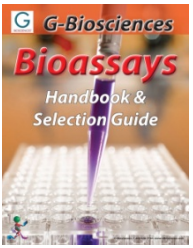
TROUBLESHOOTING:

Issue	Suggested Cause	Solution
Lower than expected readings	Wavelength used is incorrect	Measure at 660nm, or between 645-670nm
A precipitate is seen in the assay tubes/ wells	Samples incubated with reagent for more than 5 minutes	Use a 5 minute incubation
		Mix samples by pipetting and read immediately
	DNA and/or RNA are present in the samples	Add Triton® X-100 to a final concentration of 0.8%
Blank is >0.25	Interfering agents present	See Table 2 for suggested concentrations
	Incorrect storage temperature for RED 660™ Protein Assay Reagent	Store at room temperature
Assay color is darker than expected	Protein concentration too high	Dilute samples

RELATED PRODUCTS

Download our Protein Assays or Bioassay Handbook





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