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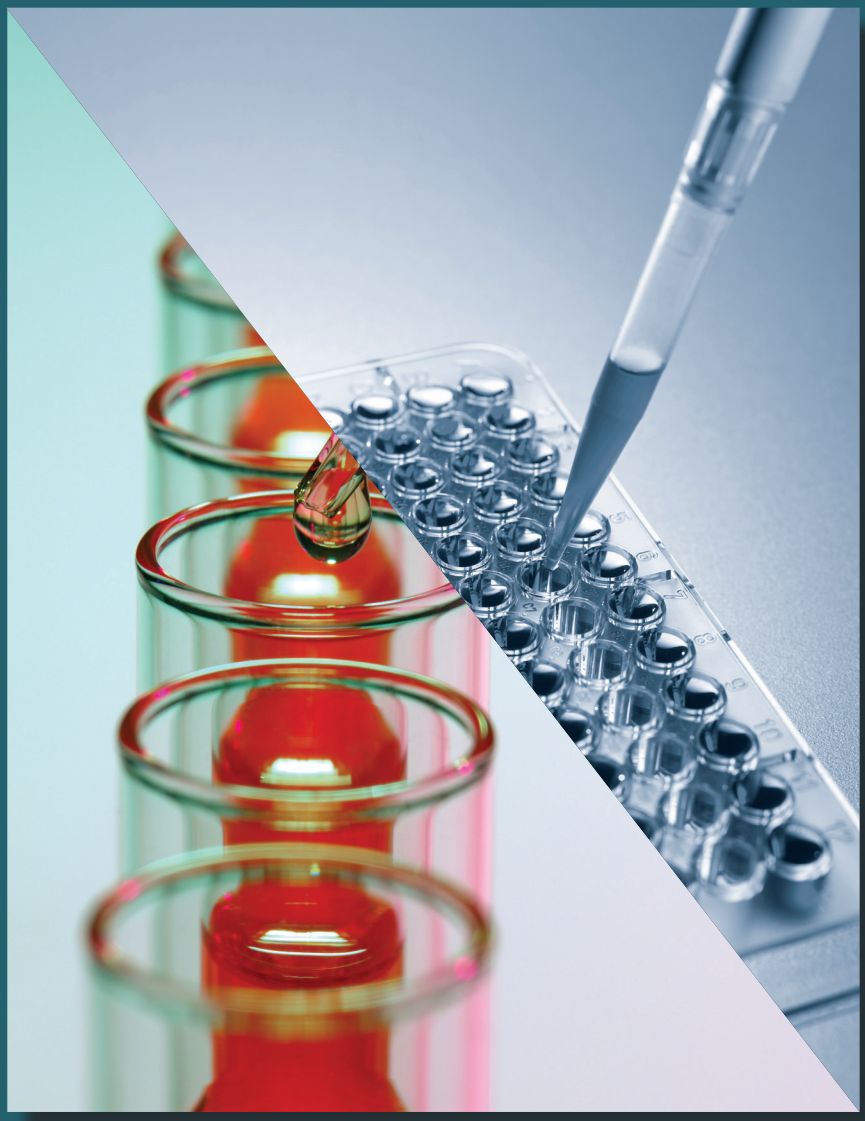

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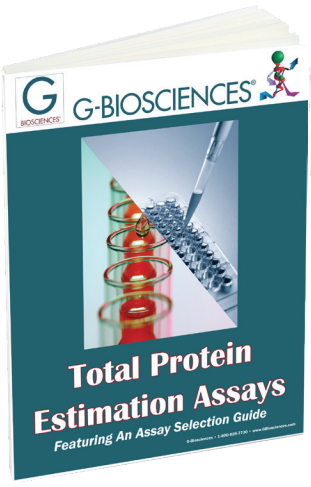
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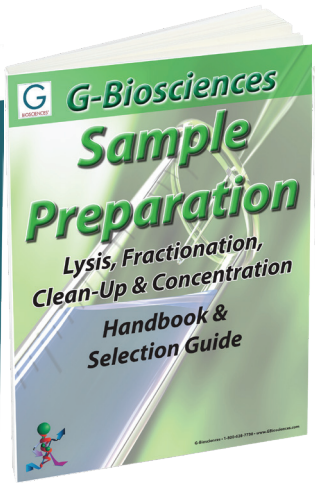
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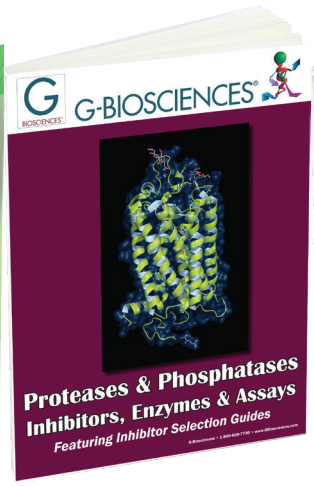
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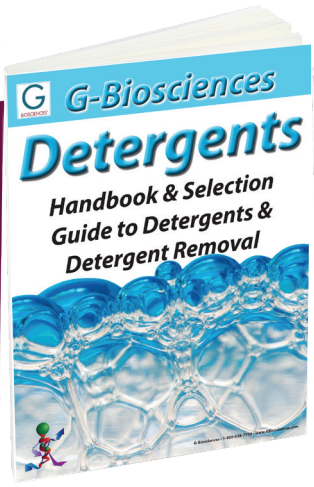
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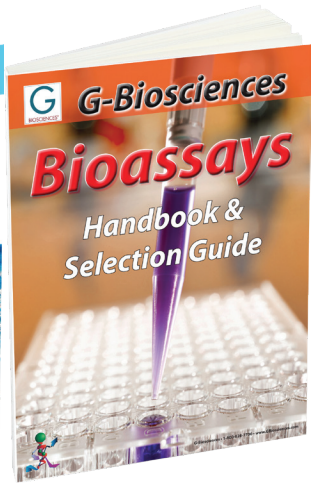
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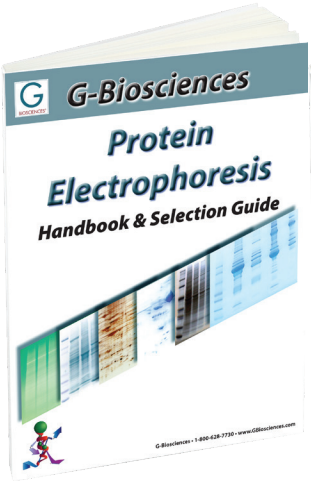
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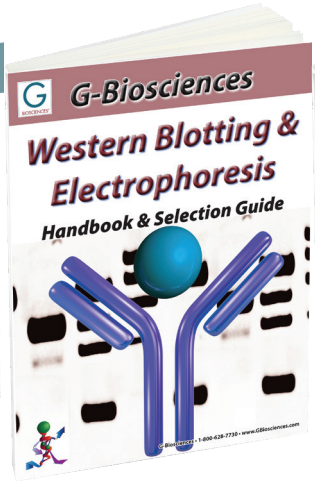
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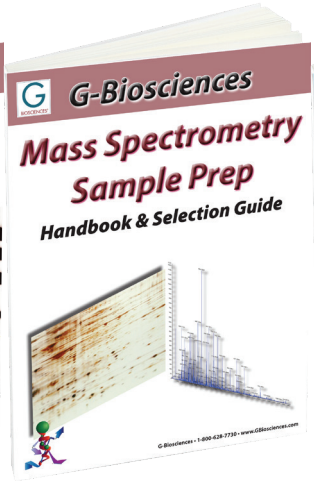
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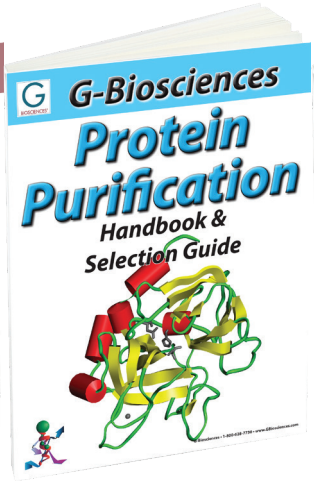
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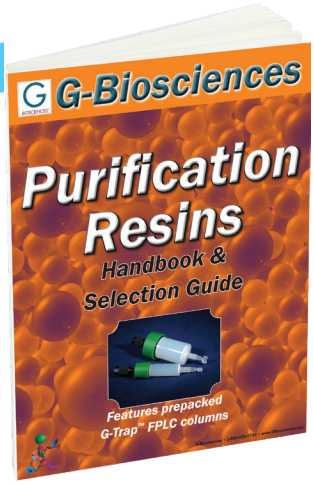
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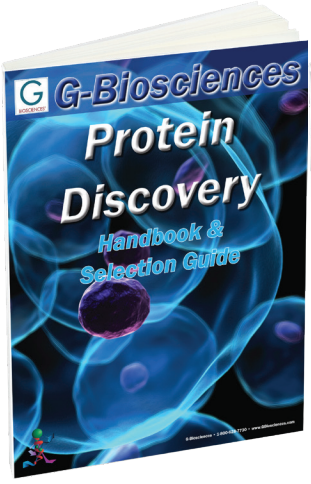
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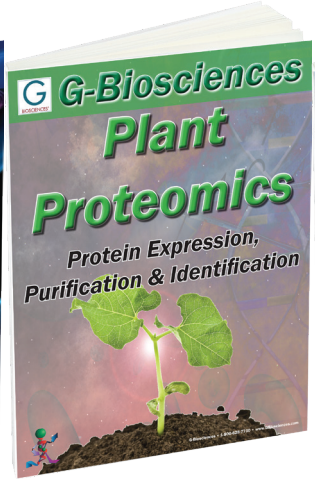
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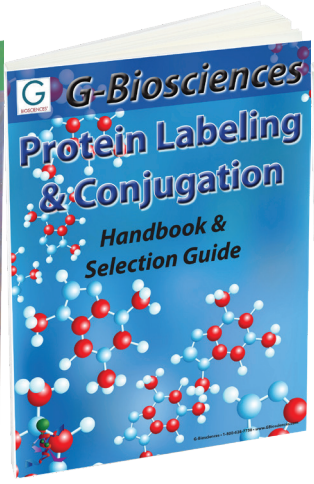
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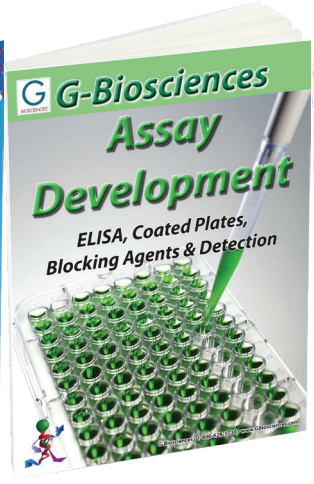
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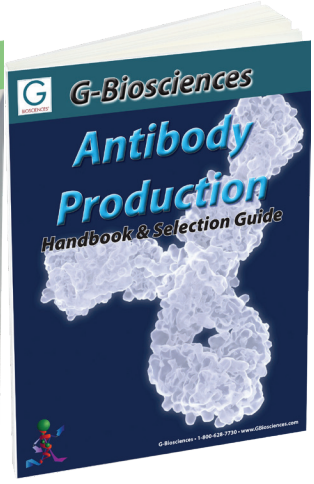
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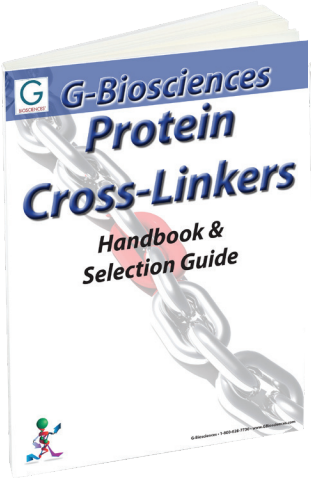
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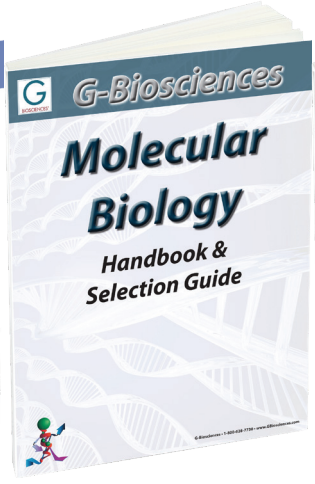
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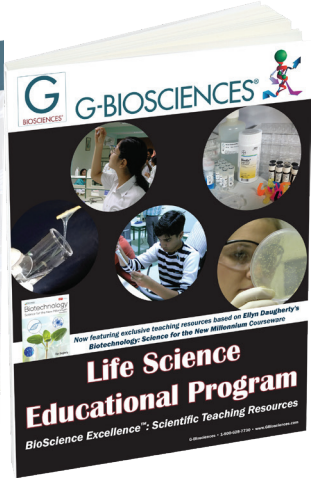
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Introduction

Protein assays are one of the most widely used methods in life science research. Estimation of protein concentration is necessary in protein purification, electrophoresis, cell biology, molecular biology and other research applications. Although there are a wide variety of protein assays available, none of the assays can be used without first considering their suitability for the application. Each assay has its own advantages and limitations and often it is necessary to obtain more than one type of protein assay for research applications.

G-Biosciences offers assays that are enhancements of dye binding protein assays, protein assays based on copper ions, or a novel test strip and spot application assay.

This guide is designed to help researchers select the most appropriate assay for their application.

Dye Binding Assays

The dye binding protein assay is based on the binding of protein molecules to Coomassie dye under acidic conditions. The binding of protein to the dye results in spectral shift, the color shifts from brown (Amax= 465nm) to blue (Amax= 610nm). The change in color density is read at 595nm and is proportional to protein concentration. The basic amino acids, arginine, lysine and histidine play a role in the formation of dye-protein complexes color. Small proteins less than 3kDa and amino acids generally do not produce color changes.

RED 660™, CB™ CB-X™ and DCB™ protein assays are dye binding protein assays and FluroRed 600 protein assay is a fluorescent dye binding assay. RED 660™ protein assay is a proprietary dye-metal complex assay.

SPN™ and SPN™-htp protein assays are spin column format dye binding assays.

Copper Ion Based Assays

In the copper ion based protein assays, the protein solution is mixed with an alkaline solution of copper salt. Under alkaline conditions, cupric ions (Cu^{2+}) chelate with the peptide bonds resulting in reduction of cupric (Cu^{2+}) to cuprous ions (Cu^{+}). If the alkaline copper is in excess over the amount of peptide bonds, some of the cupric ions (Cu^{2+}) will remain unbound to the peptide bonds and are available for detection. Protein assays based on copper ions can be divided into two groups, assays that detect reduced cuprous ions (Cu^{+}) and that detect unbound cupric (Cu^{2+}) ions.

The cuprous ions are detected either with bicinchoninic acid (BCA) or Folin Reagent (phosphomolybdic/ phosphotungstic acid). Cuprous ions (Cu^{+}) reduction of BCA Reagent produces a purple color that can be read at 562nm. The amount of color produced is proportional to the amount of peptide bonds, i.e. size as well as the amount of protein/peptide.

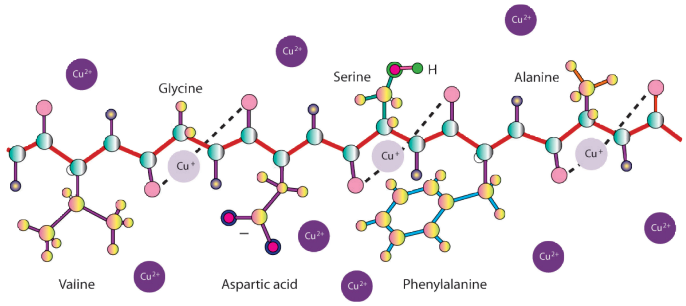

Figure 1: The interaction of copper ions with proteins.

The presence of tyrosine, tryptophan, cysteine, histidine and asparginine in protein contributes to additional reducing potential and enhances the amount of color produced. Hence, the amount of blue color produced is dependent on the composition of protein molecules. The reaction of cuprous ions (Cu^{+}) with the bicinchoninic acid and color production is similar to that of Folin Reagent.

In the assays based on the detection of unbound cupric ions, the protein solution is mixed with an amount of alkaline copper that is in excess over the amount of peptide bond. The unchelated cupric ions are detected with a color-producing reagent that reacts with cupric ions. The amount of color produced is inversely proportional to the amount of peptide bond.

Non-Interfering™ (NI™) Protein Assay is based on the detection of unbound cupric ions (Cu^{2+}) under alkaline condition. Three BCA (bicinchoninic acid) assays are offered. The ML (Modified Lowry) and CL (Compatible Lowry) are both copper ion based assays.

Test Strip Based Protein Assay

This is in effect a chromatographic capture method where the flat surface of the test strip acts as the solid matrix or support. Protein solution is applied on a specific protein binding test strip by point of contact capillary action. Under a specific buffer condition, as the protein enters into the matrix of the test strip, it binds instantly and saturates as the protein solution diffuses into the test strip in a circular manner. A circular protein imprint is produced which is developed into visible protein spots with a protein specific dye. The diameter of the protein spot is proportional to protein concentration. Measuring the protein spot diameter with a measuring gauge, the amount of protein can be estimated.

dotMETRIC™ is based on the use of test trips and spot application for protein estimation.

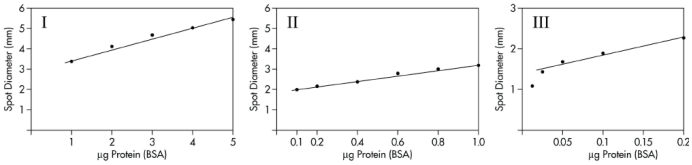

Figure 2: The linear relationship of protein BSA concentration with the protein spot diameters.

PROTEIN ASSAY SELECTION

The nature of the protein sample is by far the most important consideration for protein assay selection. If the protein sample is in a dry and solid form, it can be easily solubilized in a protein assay compatible buffer. Unfortunately, the majority of protein samples are processed and complex solutions that contain many non-protein, interfering agents. Apart from the nature of the protein sample there are other considerations that will affect the quality of protein estimation. The following section deals with many of the issues that effect the accuracy and sensitivity of protein assays.

Interfering Agents

Proteins are complex polymers of amino acids with numerous modifications and structural variations and hence require endless varieties of chemical agents for stability and analysis. The presence of non-protein agents in protein solutions creates challenges for protein assays. Protein solutions containing reducing agents, metal chelating agents, dyes, amines, and sugars, cannot be estimated with the protein assays based on copper ions. On the other hand, protein solutions containing surfactants (detergents) interfere with the dye based protein assays. The best protein estimation is possible with assays that either substantially removes non-protein agents from the protein solutions or the methods that circumvent the interfering affects of non-protein agents present in the protein samples.

Non-Interfering™ (NI™), CB-X™, SPN™, SPN™-htp and CL (Compatible Lowry) protein assays are designed to first remove non-protein agents from the protein solutions. RED 660™ and Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay are designed to be more resistant to detergents and reducing agents respectively. DCB™ and ML (Modified Lowry) protein assays are compatible with detergents.The dotMETRIC™ protein assay on the other hand is designed to circumvent the interfering effects of non-protein agents in the protein solution.

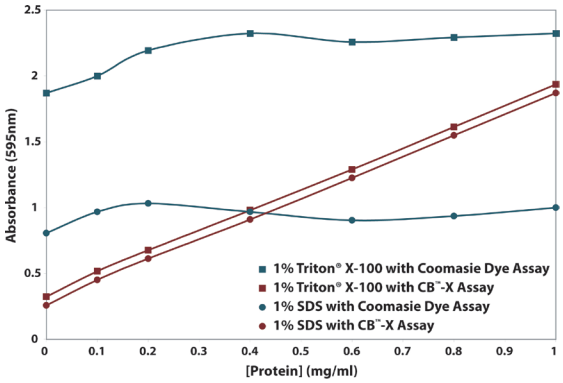

Figure 3: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A linear response to increasing protein concentration was visualized, indicating no interference by the detergents.

Sample Preparation

For protein analysis, samples must be in a solubilized form. Solid samples must be first solubilized in an appropriate buffer, preferably containing non-interfering agents. When working with cells and tissues, the first step is to disaggregate the sample using a grinding tool and then solubilize it in a lysis buffer. The soluble protein is collected either by centrifugation or filtration. The lysis buffer should preferably be free from agents that may interfere with protein assays. If the protein solubilization buffer contains interfering agents, it must be removed by dialysis. Alternatively, use a protein assay method that is not affected by the presence of non-protein agents.

Assay Sensitivity and Sample Size

For hard to obtain samples, the size of protein samples sacrificed in protein estimation becomes a critical consideration. Most colorimetric protein assays require at least 0.5µg proteins for a reliable estimation. If the protein estimation is made using a duplicate set of samples, then the estimation will require the sacrifice of at least 1µg protein in each sample. The methods that require the lowest amount of protein sample for a reliable estimation of protein will offer an advantage over other methods.

Protein dotMETRIC™ assay requires the lowest amount of protein over all other protein assays in use. A protein measurement can be performed with as little as 25-30ng proteins each sample.

Dilute Protein Sample

Since most colorimetric protein assays require at least 0.5µg proteins for a reliable estimation, dilute protein solutions require a larger volume to reach the limit of detection for the protein assay. Use of samples >10% of the total assay volume tends to interfere with most assays. For example, in the dye based protein assays, if the sample volume increases over 10% of the total assay reaction volume, the linearity of assay begins to break down due to shift in reaction pH created by large sample volume. Protein assays that concentrate the samples, including dilute samples, as a normal course of assay procedure have an advantage. Dilute protein samples can be assayed without any adverse effect on the quality of protein estimation or requiring any modification to account for dilute protein sample.

The NI™ -Protein Assay and the CB-X™ both, as a normal course of assay protocol, concentrate the protein samples and therefore even dilute protein solutions can be assayed without any concern.

Micro Bicinchoninic Acid (BCA) Protein Assay is designed for dilute protein samples, but requires larger sample volumes.

Time Consideration & Assay Time

The amount of time taken to perform a protein assay will depend on the complexity of the sample and the assay method. Protein assays that use standard plots or curves are the most time consuming. Protein samples containing interfering agents are time consuming as the interfering agents need to be removed. Protein assays that are not reliant on standard plots allow for quick protein concentration determination and are ideal when there are a limited number of samples for protein estimation.

Most dye based protein assays and copper ion based assays require preparation of standard plots. Protein dotMETRIC™ protein assay and the dye binding CB-X™ and SPN™ protein assay do not require preparation of standard plots as they use premade charts or tables saving time and money.

Protein Standards

The most reliable protein estimation is performed using a reference or a protein standard that has properties similar to the protein being estimated. Often it is difficult to find a protein standard with similar properties to the sample being analyzed. As a result, it has become acceptable to use readily available proteins such as bovine serum albumin (BSA) and gamma globulin as standards. Using either the BSA or the bovine γ-globulin (IgG) as reference proteins, most protein assay methods show significant protein-to-protein variation. Protein assays independent of the use of protein standards will show little or no dependency on the choice or the use of protein standards.

The test strip based dotMETRIC™ protein assay and the dye binding CB-X™ and SPN™ protein assay do not require the use of a protein standard.

Protein-to-Protein Variations

Dye based protein assays show the largest protein-to-protein variation and in some cases (i.e. gelatin), these assays show no protein response as no protein-dye complex is formed.

Assays involving the reduction of cuprous ions to cupric ions have significant protein-to-protein variation.

Assays in which unbound cupric ions are assayed show significantly lower protein-to-protein variations, as measuring free and unbound cupric ions is significantly independent of protein primary structure.

The test strip based dotMETRIC™ protein assay, based on the chromatographic capture of the proteins, is independent of the primary structure of the protein and hence shows little or no protein-to-protein variation.

The NI™-Protein Assay, based on the detection of unbound cupric ions, and the dotMETRIC™, based on chromatographic capture of proteins, are both independent of protein-to-protein variation.

Instrumentation Requirements

Most protein assays require use of colorimeters or spectrophotometers. For high-throughput applications, multi-well titer plates are more convenient, however, not every protein assay can be adapted to run in titer plates.

The dotMETRIC™ assay does not require instrumentation and can therefore be used either in the laboratory or in the field.

G-Biosciences offers protein assays and accessories for a wide variety of applications requiring the estimation of protein concentration. We offer colorimetric protein assays, single tube assays, as well as test strip based assays for rapid analysis. These assays are suitable even for the most demanding research applications and are:

CB-X™

One Assay for All Jobs

A major problem for researchers is to select a protein assay from the vast selection on the market that is compatible with their protein sample.

CB-X™ Protein Assay eliminates this problem as it is designed to be compatible with all commonly used buffers and conditions in protein isolation, storage and assays.

For protein samples in simple, uncomplicated aqueous buffers CB-X™ is a highly sensitive, single reagent assay that can be performed in 5 minutes. CB-X™ Protein Assay uses a protein dye that is an improvement on the Bradford Coomassie dye.

For complicated protein samples CB-X™ Protein Assay is supplied with reagents to clean up the samples and remove all reagents and chemicals in a single step that interfere with accurate protein estimation. These reagents include detergents, chaotropes, reducing agents, alkylating agents, sugars, high salt concentrations, buffering agents and chelating agents . The clean up stage and subsequent protein assay is performed in a single tube to ensure no protein loss and to maintain the accuracy of the assay.

DETERGENTS		REDUCING AGENTS	
Brij® 35	2%	2-Mercaptoethanol	1M
CHAPS	2%	DTT	1M
CHAPSO	2%	**CHAOTROPES**	
Nonidet® P-40	2%	Guanidine-HCl	6M
SDS	2%	Urea	6M
Triton® X-100	2%	**SALTS**	
Tween® 20	2%	Ammonium Sulfate	1M
Deoxycholate	0.1%	**MISCELLANEOUS**	
SUGARS		EDTA	0.1M
Glucose	1M	HEPES	0.1M
Sucrose	25%	MES	0.7M

Table 1: CB-X™ Protein Assay is compatible with many interering agents.

If the protein sample does not contain interfering agents then a straightforward single reagent assay is performed to give a linear response. If interfering agents are present or if artifactual results are produced then the protein samples are treated in a single step with the clean up reagents and the protein is assayed generating a linear response.

CB-X™ Protein Assay is supplied with lot specific CB-X™ Tables. These allow researchers to perform single protein clean ups, subsequent assays and then look up their absorbance in the CB-X™ Table to find the protein concentration. The CB-X™ Table eliminates the need for multiple protein standards and saves considerable time and effort. The CB-X™ Table is prepared with a complex protein mixture that compares well with proteins from mammalian, plant, bacteria and yeast sources.

The assay is supplied with a BSA protein standard or a non animal protein standard with Cat. # 786-12X or 786-894 respectively for generating curves when using CB-X™ Assay Dye alone or for researcher's who prefer to generate their own standard curve or their own CB-X™ Table per their specific conditions.

The CB-X™ Protein Assay is reliable over the range of 0.5-50µg per assay. The regular size kit contains enough CB-X™ Assay Dye for 500 protein assays and enough clean up reagents for 250 clean ups.

Cat. No.	Description	Size
786-12X	CB-X™ Protein Assay with Albumin Standard	500 Assays
786-12XT	CB-X™ Protein Assay Trial	10 Assays
786-894	CB-X™ Protein Assay with Non Animal Protein Standard	500 Assays
786-11X	CB-X™ Protein Assay (No BSA Standard)	500 Assays

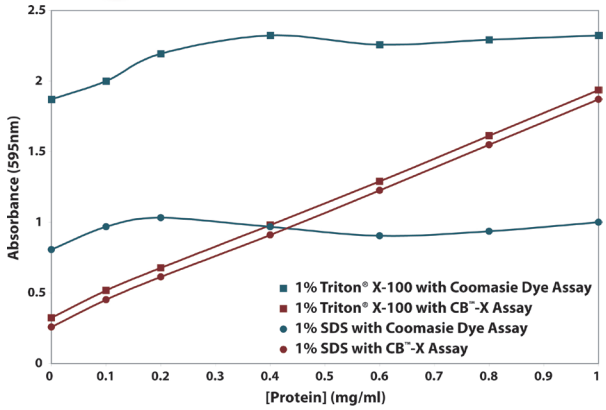

Figure 4: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A linear response to increasing protein concentration was visualized, indicating no interference by the detergents.

FEATURES

- 0.5-50µg Linear Response
- Rapid Precipitation & Color Development
- Long shelf life, stable for 12 months
- High Reliability and Reproducibility

APPLICATIONS

CB-X™ has been used in a wide array of techniques and applications including

- Protein estimation in protein purification, electrophoresis, immunoanalysis, cell biology, molecular biology and other research applications
- Protein samples containing common laboratory agents
- Detergent solubilized membrane proteins
- Dilute protein solutions

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More citations available at www.GBiosciences.com

CB™ Protein Assay

A Coomassie Dye Based Protein Assay

It is 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. 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. The change in color density is proportional to protein concentration. Protein estimation can be performed using as little as 0.5µg protein.

CB™ Protein Assay is supplied with a simple to follow protocol and a ready to use reagent that does not require prefiltering or dilution. Simply mix the protein solution with CB™ Protein Dye and read optical density.

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.

Note: The Coomassie dye based assay is not suitable if the protein solution contains higher than recommended concentration of detergents or other agents.

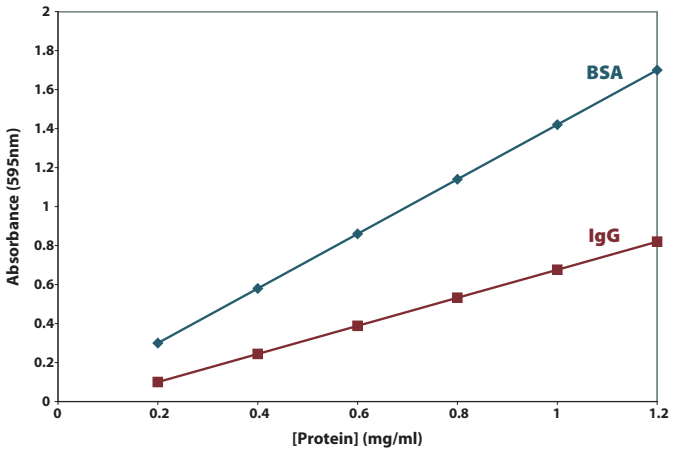

Figure 5: Linear Response and Protein-to-Protein variation with CB™ Protein Assay.

FEATURES

- Sensitivity: Linear responses over the range of 0.5µg-50µg protein
- Flexible Protocols: Suitable for tube or Titer plate assays
- Ready to use assay reagents and no preparation required
- Long shelf life, stable for 12 months

APPLICATIONS

- Suitable for non-detergent solubilized proteins
- Protein estimation in protein purification, electrophoresis, cell biology, molecular biology, and other research applications
- Suitable for protein samples containing common laboratory agents

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.

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More citations available at www.GBiosciences.com

Cat. No.	Description	Size
786-012	CB™ Protein Assay with Albumin Standard	500 Assays
786-893	CB™ Protein Assay with Non Animal Protein Standard	500 Assays

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	0.06%
Ethanol	10%	tRNA	0.35mg/ml
Glucose	1M	Tween® 20	0.03%
Glycerol	10%	Urea	3M

Table 2: A selection of compounds and the maximum concentrations compatible with CB™ Protein Assay.

DCB™ Protein Assay

Detergent-compatible Bradford assay

DCB™ Protein Assay is a Coomassie Dye (Bradford) based detergent compatible assay. The Assay contains proprietary reagents suitable with 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.

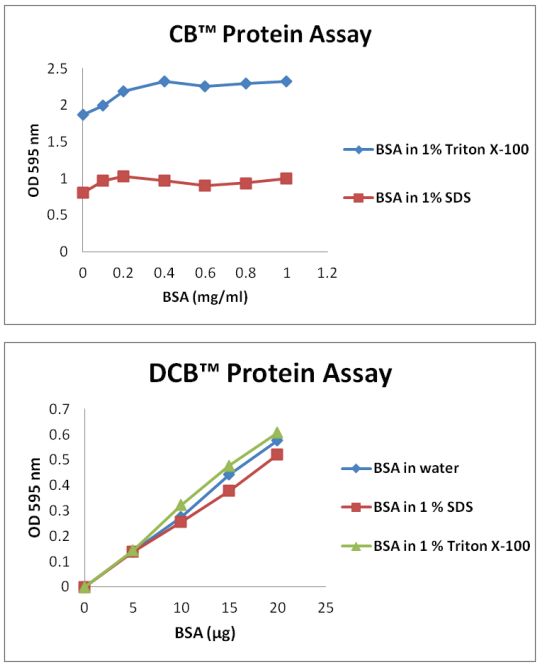

Figure 6: BSA standard with and without detergents compared with CB™ Protein Assay (top) and DCB™ Protein Assay (bottom).

Compounds	Conc.
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%

Table 3: A selection of compounds and buffers with their maximum concentrations that are compatible with the RED 660™ Protein Assay.

FEATURES

- Compatible with detergents containing samples.
- Rapid: The reaction reaches optimal within 5 minutes.
- Sensitivity: can detect as low as 0.5µg protein with detection range from 1 to 1000 µg/ml.
- Available in 500 test tube or 2500 micowell assays.

APPLICATIONS

- DCB™ Protein Assay is suitable for detergent containing solubilization and lysis buffers.
- For protein estimation in protein purification, electrophoresis, cell biology, molecular biology, and other research applications.
- Suitable for protein samples containing common laboratory agents.

Cat. No.	Description	Size
786-1594T	DCB™ Protein Assay	15 assays
786-1594	DCB™ Protein Assays with Albumin Standard	500 assays

FluroRed 600 Protein Assay

FluroRed 600 Protein Assay is fluorescent dye based assay with higher detection range and low interference from common lab reagents when compared to colorimetric protein assay. FluroRed 600 Protein Assay is comprised of fluorescent dye which has different absorption and emission spectrum in conjugated and non-conjugated form. The dye bind to the proteins through their amine groups and upon binding the absorption and emission spectrum shifts to 500/600nm (Ex/Em). The absorption can be read anywhere between 490±10 nm and emission between 600±10 nm.

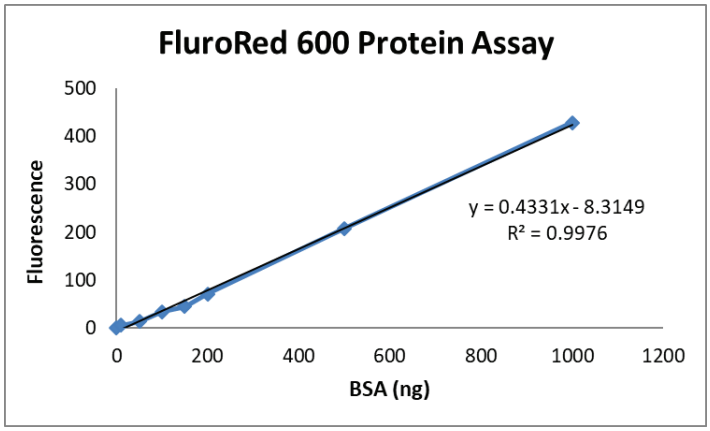

Figure 7: Standard curve generated with FluroRed Protein Assay.

FEATURES

- High efficiency: no interference from free dye in solution and low interference from common lab reagents
- Sensitivity: can detect as low as 50 ng of protein.
- Simple, easy-to-use and rapid assay
- Available in 100 and 500 micowell assays format.

APPLICATIONS

- High sensitive detection of protein concentration via fluorescence.
- Ideal for protein estimation in protein purification, electrophoresis, cell biology, molecular biology, and other research applications.
- Ideal for protein samples containing common laboratory agents.

Cat. No.	Description	Size
786-1595	FluroRed 600 Protein Assay	100 microwell assays
786-1596	FluroRed 600 Protein Assay	500 microwell assays

RED 660™ Protein Assay

Detergent & Reducing Agent Compatible

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.

RED 660™ Protein Assay is compatible with most detergents and its compatibility can be furthered enhanced with Neutralizer™. Neutralizer™ is a unique chemical that sequesters ionic detergents, including SDS, allow the solution to be compatible with the RED 660™ Protein Assay. The Neutralizer™ can also be used with Laemmli loading buffer.

INTERFERENCE TO PROTEIN ASSAY

Agents compatible with the RED 660™ Protein Assay are shown and acceptable concentration of reagents for standard protocols are listed. 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™.

FEATURES

- Linear response 0.5µg-20µg protein
- **Rapid:** Single reagent assays
- **Versatile:** Compatible with higher range of detergents and reducing agents
- **Linear:** Perfect linear standard curves compared to other protein assays

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. 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	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 4: Protein-to-protein variation.

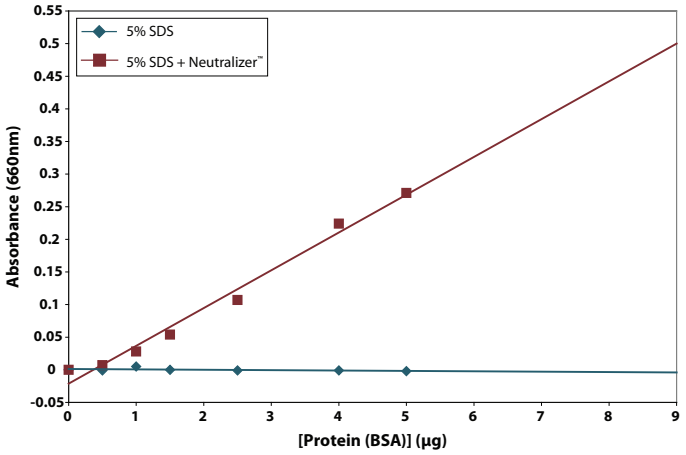

Figure 8: Presence of Neutralizer™ overcomes 5% SDS interference.

Compounds	Conc.	Compounds	Conc.
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™	Dil. 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	Yes	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 5: A selection of compounds and buffers with their maximum concentrations that are compatible with the RED 660™ Protein Assay.

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2. Aykul, S. and Martinez-Hackert, E. (2016) Transforming Growth Factor-β Family Ligands Can Function as Antagonists by Competing for Type II Receptor Binding.J. Biol. Chem. 2016; 291:10792-10804.

More citations available at www.GBiosciences.com

Cat. No.	Description	Size
786-676	RED 660™ Protein Assay	500 standard/ 2,500 microwell
786-899	RED 660™ Protein Assay with Non Animal Protein Standard	500 Assays/ 2,500 Micro-assays
786-603	Neutralizer™	10 vials
786-604	Neutralizer™	12.5g
786-673	Neutralizer™	5 vials

SPN™ Protein Assay

An Ultra Sensitive Spin Format Protein Assay!

A novel protein assay that is suitable for a single sample or high throughput protein estimation. The SPN™ and SPN™-htp protein assays are rapid assays that are suitable for as little as 0.5µg protein and are resistant to interference from common laboratory agents. The assays are excellent for the rapid determination of protein samples in Laemmli or other SDS-PAGE loading buffers.

These protein assays are based on the quantitative capture of protein on to a proprietary matrix. The bound protein is treated with a protein specific dye that associates proportionally with the protein. The protein bound dye is eluted and measured to determine the protein concentration. For increased efficiency, each assay is supplied with its own reference data for rapid calculation of the protein concentration without a need for a set of protein standards.

SPN™ PROTEIN ASSAY

A fast and efficient spin column assay. Add the protein sample to the SPN™ spin columns and wash to remove non-protein agents, including detergents and chaotropes. Next, add the protein dye and spin to remove free dye. After a second brief wash, elute the protein bound dye with the supplied elution buffer and measure the optical density of the dye. The concentration of the protein is determined by comparing the optical density data to the supplied reference data. No protein standards are required.

Figure 9: The SPN™ spin columns

SPN™ -HTP PROTEIN ASSAY

The SPN™-htp protein assay, based on our SPN™ method, has been modified for use in high throughput protein concentration determination. The SPN™-htp protein assay format is suitable for semi-automative assays that utilize a vacuum manifold or a fully automated robotic plate format in an online configuration; also fully compatible with 96-well centrifuge adaptors. The assay can be performed with or without a set of known protein standards and shows a linear response between 0.5-10µg protein.

Figure 10: The SPN™-htp protein assay.

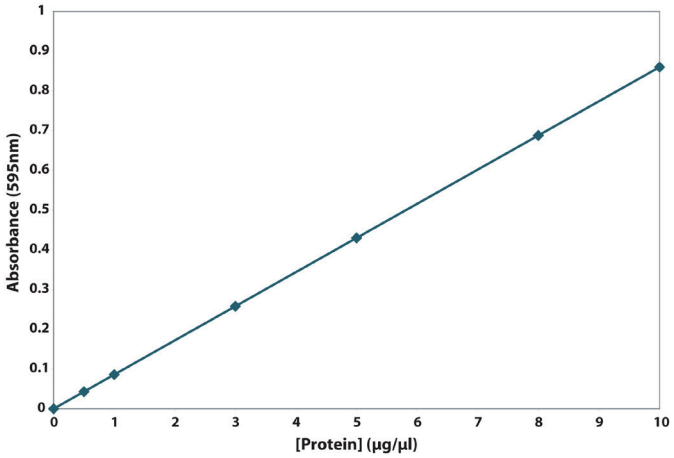

Figure 11: Standard Calibration Plot for SPN™ Protein Assay.

FEATURES

- Reliable linear response over the range of 0.5-10µg
- Manual, semi-automatic or fully automated compatible
- Unaffected by non-protein chemicals and agents
- Rapid assay; protein standards not required
- No toxic agents used, laboratory & environment safe

APPLICATIONS

- Rapid protein estimation
- Measure protein concentration in gel loading buffer

CITED REFERENCES

1. Riccio, A.M. et al (2017) Galectin-3: an early predictive biomarker of modulation of airway remodeling in patients with severe asthma treated with omalizumab for 36 months. Clin Transl Allergy. DOI: 10.1186/s13601-017-0143-1

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Compounds	Concentration
Ammonium Sulfate	20%
Brij® 35	2%
Brij® 58	2%
CHAPS	2%
CHAPSO	2%
DTT	1M
Guanidine.HCl	6M
Mercaptoethanol	1M
Non-Detergent Sulfobetaine 201	2%
Potassium Chloride	50mM
Sodium Chloride	0.1M
Sodium Deoxycholate	1%
Triton® X-100	2%
Tween® 20	1%
Urea	6M

Table 6: Compatible substances for SPN™ Protein Assay.

Cat. No.	Description	Size
786-020	SPN™ Protein Assay Kit	50 Assays
786-021	SPN™-*htp* Protein Assay Kit	5 x 96 assay plates

NI™ (Non-Interfering™) Protein Assay

Unaffected by interfering agents

A highly sensitive, colorimetric protein assay that overcomes interference of common laboratory agents present in protein solutions and shows minimal protein-to-protein variation.

The assay is unaffected by the presence of common laboratory agents, such as reducing agents, chelating agents, detergents, amines, sugars, chaotropes, salts, drugs, antibiotics, cobalt and other common laboratory agents. The NI™ Protein Assay is composed of two simple steps

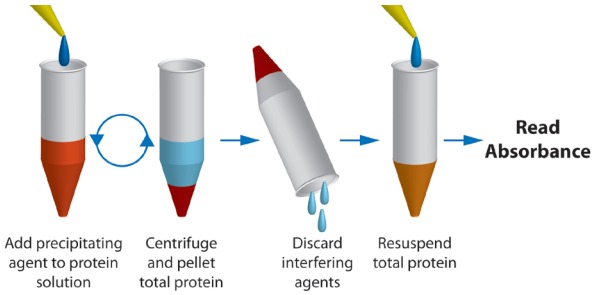

Figure 12: The NI™ Protein Assay Scheme

Universal Protein Precipitating Agent (UPPA™) is added to the protein solutions to rapidly precipitate total protein. Protein is immobilized by centrifugation and interfering agents in the supernatant are discarded.

Protein concentration is assayed by mixing with an alkaline copper solution; the copper ions bind to the peptide backbone and the assay measures the unbound copper ions. The assay is independent of protein side chains minimizing protein-to-protein variation. The color density is inversely proportional to the amount of protein.

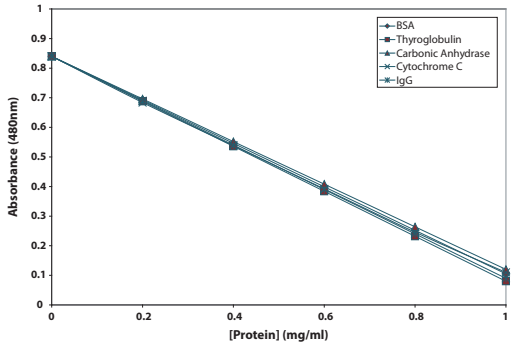

Figure 13: NI™ Protein Assay shows minimal protein-to-protein variation. BSA, thyroglobulin, carbonic anhydrase, cytochrome C and bovine immunoglobulin G were assayed for protein-to-protein variation. The proteins produced identical color and slope in the range of 0.5µg-50µg, giving an average ratio of 1.01.

Buffer Composition
4M urea, 1% SDS, 10mM EDTA, 0.8% 2-Mercaptoethanol
6M urea, 2M thiourea, 4% CHAPS
6M urea, 2M thiourea, 4% Nonidet® P-40
1% Sarcosyl, 0.8% 2-Mercaptoethanol, 4M guanidine thiocyanate, 10 mM EDTA
6M urea, 2M thiourea, 2% CHAPS, 2% ND SB 201
6M urea, 2M thiourea, 2% CHAPS, 2% SB 2 10

Table 7: NI™ Protein Assay is compatible with strong chaotropic extraction buffers.

FEATURES

- Linear response 0.5µg-50µg protein
- Small sample requirement, only 1-50µl
- Unaffected by non-protein chemicals and agents
- Protocol time: ~30 minutes
- Long Shelf Life, stable for 1 year

APPLICATIONS

- Estimate protein during protein purification, electrophoresis, cell biology, molecular biology, and other research applications
- Suitable for protein samples containing common laboratory agents, such as reducing agents (β-mercaptoethanol, dithiothreitol (DTT)), chelating agents (EDTA), detergents, amines (Tris), sugars and many other agents
- Suitable for samples containing chaotropic agents such as urea, thiourea, guanidine hydrochloride, guanidine thiocyanate, ammonium sulfate, drugs, antibiotics, cobalt, and numerous other agents and extraction buffers
- Suitable for determination of protein concentration in cellular fractions, tissue & cell lysates and chromatography purification fractions
- Suitable for dilute protein solutions

Compounds	Conc.	Compounds	Conc.
Ammonium sulfate	40%	N-Octyl glucoside	0.5%
Brij® 35	1%	Phosphate buffer	0.2M
CHAPS	1%	Sarcosyl	1%
CHAPSO	1%	Sodium azide	0.1M
CTAB	1M	Sodium dodecyl sulfate	1%
Digitonin	0.3%	Sucrose	30%
DTT	10mM	TCEP	15mM
EDTA	10mM	Thesit®	2%
Glycerol	30%	Thiourea	2M
Guanidine.HCl	6M	Tris	0.2M
Guanidine thiocyanate	6M	Triton® X-100	3%
HEPES	0.1M	Triton® X-114	1%
Iodoacetamide	15mM	Tween® 20	2%
2-mercaptoethanol	0.5%	Urea	8M

Table 8: A selection of compounds and the maximum concentrations that are compatible with the NI™ Protein Assay.

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More citations available at www.GBiosciences.com

Cat. No.	Description	Size
786-005	NI™ (Non-Interfering™) Protein Assay Kit with Albumin Standard	500 Assays
786-896	NI™ (Non-Interfering™) Protein Assay Kit with Non Animal Protein Standard	500 Assays

Bicinchoninic Acid Protein Assay

Sensitive, Detergent Compatible Assay

The Bicinchoninic Acid (BCA) Protein Assay is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions. The Bicinchoninic Acid (BCA) Protein Assay primarily relies on two reactions. Firstly, the peptide bonds in the protein sample reduce Cu^{2+} ions, in a temperature dependent reaction, from the copper solution to Cu^{+}. The amount of Cu^{2+} reduced is proportional to the amount of protein present in the solution. Next, two molecules of bicinchoninic acid (BCA) chelate with each Cu^{+} ion, forming a purple-colored product that strongly absorbs light at a wavelength of 562nm that is linear for increasing protein concentrations between the range of 0.02-2mg/ml. The amount of protein present in a solution can be quantified by measuring the absorption spectra and comparing with protein solutions with known concentrations.

Suitable for quantifying protein solutions in 1ml assays or in microwells.

Compounds	Conc.	Compounds	Conc.
2-Mercaptoethanol	0.01%	Iron	Incompatible
Ammonium sulfate	1.5M	Lipids	Incompatible
Ascorbic acid	Incompatible	N-Octyl Glucosidase	5%
Brij® 35	5%	Phenol red	Incompatible
Catecholamines	Incompatible	Phosphate buffer	0.1M
CHAPS	5%	SDS	5%
CHAPSO	5%	Sodium azide	0.2%
Creatinine	Incompatible	Sodium Chloride	1M
Cysteine	Incompatible	Sucrose	40%
Deoxycholic acid	5%	Tris.HCl	0.25M
DTT	1mM	Triton® X-100	5%
EDTA	10mM	Triton® X-114	1%
EGTA	Incompatible	Tryptophan	Incompatible
Glycerol	10%	Tyrosine	Incompatible
Guanidine.HCl	4M	Tween® 20	5%
HEPES	0.1M	Urea	3M
Hydrogen peroxide	Incompatible	Uric acid	Incompatible
Hydrazides	Incompatible	Zwittergent® 3-12	1.0%
Imidazole	0.05M		

Table 9: Compatible substances for Bicinchoninic Acid (BCA) and Micro Bicinchoninic Acid (BCA) Protein Assay.

FEATURES

- Sensitive colorimetric assay
- Linear range of 20-2,000µg/ml
- Compatible with wide range of ionic and non-ionic detergents
- 1ml cuvette or micro well compatible

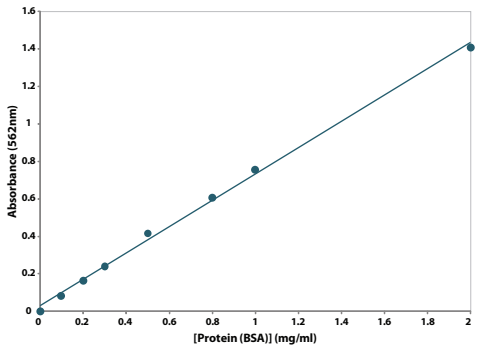

Figure 14: Bicinchoninic Acid (BCA) Protein Assay Standard Curve.

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Micro BCA Protein Assay

For Dilute Protein Samples

The Micro Bicinchoninic Acid (BCA) Protein Assay is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions and is a modification of the Bicinchoninic Acid (BCA) Protein Assay for dilute protein samples (0.5-20µg/ml). The Micro Bicinchoninic Acid (BCA) Protein Assay is suitable for quantifying protein solutions in 1ml assays or in micro-wells and is for 500 x 1ml assays or >3,300 x Micro-well assays.

Bicinchoninic Acid Reducing Agent Compatible Protein Assay

Reducing Agent Compatible BCA Assay

The Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay is supplied with the Reducing Agent Compatibility Agent (RACA) that modifies reducing agents to limit their effect on the reduction of the assay's copper ions, preventing inhibition of the assay. The use of RACA allows for samples containing up to 5mM DTT, 10mM TCEP or 35mM ß-mercaptoethanol.

The BCA Reducing Agent Compatible Protein Assay is suitable for quantifying protein solutions in 1ml assays. The kit is suitable for 250 x 1ml assays.

Compounds	Conc.	Compounds	Conc.
2-Mercaptoethanol	35mM	Iron	Incompatible
Ammonium sulfate	1.5M	Lipids	Incompatible
Ascorbic acid	Incompatible	N-Octyl Glucosidase	5%
Brij® 35	5%	Phenol red	Incompatible
Catecholamines	Incompatible	Phosphate buffer	0.1M
CHAPS	5%	SDS	5%
CHAPSO	5%	Sodium azide	0.2%
Creatinine	Incompatible	Sodium Chloride	1M
Cysteine	Incompatible	Sucrose	40%
Deoxycholic acid	5%	TECP	10mM
DTT	5mM	Tris.HCl	0.25M
EDTA	10mM	Triton® X-100	5%
EGTA	Incompatible	Triton® X-114	1%
Glycerol	10%	Tryptophan	Incompatible
Guanidine.HCl	4M	Tyrosine	Incompatible
HEPES	0.1M	Tween® 20	5%
Hydrogen peroxide	Incompatible	Urea	3M
Hydrazides	Incompatible	Uric acid	Incompatible
Imidazole	0.05M	Zwittergent® 3-12	1.0%

Table 10: Compatible substances for Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay.

Cat. No.	Description	Size
786-570	Bicinchoninic Acid (BCA) Protein Assay	500 x 1ml assays 2,500 x microwell assays
786-571	Bicinchoninic Acid (BCA) Protein Assay	1,000 x 1ml assays 5,000 x microwell assays
786-890	Bicinchoninic Acid (BCA) Protein Assay with Non-animal Protein Standard	500 Assays/ 2500 Micro-assays
786-891	Bicinchoninic Acid (BCA) Protein Assay with Non-animal Protein Standard	1,000 Assays/ 5,000 Micro-assays
786-572	Micro Bicinchoninic Acid (BCA) Protein Assay	500 x 1ml assays >3,300 x microwell assays
786-895	Micro Bicinchoninic Acid (BCA) Protein Assay with Non Animal Protein Standard	500 Assays/ 3,300 Micro-assays
786-573	Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay	250 x 1ml assays
786-892	Bicinchoninic Acid (BCA) Protein Assay: Reducing Agent Compatible with Non Animal Protein Standard	250 assays
786-844	BCA Solution (BCA Reagent A)	250ml
786-845	BCA Solution (BCA Reagent A)	500ml
786-846	BCA Solution (BCA Reagent A)	1L
786-847	BCA Solution (BCA Reagent A)	1gal
786-848	Copper Solution (BCA Reagent B)	25ml
786-861	Assay Buffer (Micro BCA Reagent)	250ml
786-862	Micro BCA Solution (BCA Reagent)	240ml
RC-127	Bicinchoninic Acid; BCA	5g
RC-128	Bicinchoninic Acid; BCA	25g

ML (Modified Lowry) Protein Assay

The ML (Modified Lowry) Protein Assay is based on the widely cited protein assay by Lowry et. al. (1951). The ML Protein Assay is compatible with a wide variety of detergents used in protein research in addition to other common reagents such as EDTA and Tris. The assay is supplied with either traditional bovine serum albumin (BSA) protein standard, pre-diluted BSA protein standards, non animal protein standard or bovine γ globulin protein standard.

Reagent	Compatibility	Reagent	Compatibility
Amino Acids	Compatible	NP-40	2%
Ammonium Sulfate	0.5M	Octaethyleneglycol dodecyl ether	0.2%
β-Mercaptoethanol	X	Octyl Glucoside	1%
Brij® 35	1%	Phosphate Buffer	-
Calcium Chloride	0.05M	Sarcosyl	-
CHAPS	1%	SDS	10%
CHAPSO	1%	Sodium Azide	0.05%
CTAB	-	Sodium Chloride	-
Deoxycholate	1%	Sodium Hydroxide	0.5M
Digitonin	0.3%	Sucrose	-
DTT	0.001M	TCEP	-
EDTA	0.025M	Thesit®	1%
Glycerol	-	Thiourea	-
Guanidine.HCl	0.4M	Tributylphosphine (TBP)	0.002M
Guanidine Thiocynate	-	Tris (pH 8)	0.1M
HEPES	-	Triton® X-100	1%
Hydrochloric Acid	0.5M	Triton® X-114	1%
Imidazole	-	Tween® 20	1%
Iodoacetamide	-	Urea	4M
Laemmli Buffer (w/5% β-Mercaptoethanol)	-	Zwittergent® 3-12	-

Table 11: A selection of compounds and their maximum concentrations compatible with the ML Protein Assay. -, not tested; X, not compatible.

FEATURES

- Protein quantitation in the presence of detergents
- Quick 15 minute incubation
- Increased color stability (color will not change more than 5% in 1 hour or 10% in 2 hours)
- Linear response 0.2-1.5 mg/ml
- One simple protocol
- Convenient kit provides all necessary reagents for 250 standard (5ml) assays or 1000 microtube (1.5-2 ml) assays

APPLICATIONS

- Suitable for protein samples containing common laboratory agents, such as chelating agents (EDTA), detergents, amines (Tris), sugars and many other agents.

Cat. No.	Description	Size
786-1075	ML Protein Assay with BSA Standard	1000 assays
786-1076	ML Protein Assay with Non Animal Protein Standard	1000 assays
786-1082	ML Protein Assay with Pre-Diluted BSA Standard	1000 assays
786-1083	ML Protein Assay with Bovine γ Globulin Standard	1000 assays
323C-A	Copper Solution	125ml
323R-A	Reagent D	2.5ml
336F-B	Folin's Reagent	250ml

CL (Compatible Lowry) Protein Assay

The CL (Compatible Lowry) Protein Assay is based on the widely cited protein assay by Lowry et. al. (1951). The CL Protein Assay is reducing agent compatible version, suitable for protein samples contain reducing agents (such as dithiothreitol (DTT), ß-mercaptoethanol and TCEP) and a wide variety of commonly used laboratory agents that are known to interference with Lowry's protein assays. The CL Protein Assay uses G-Biosciences' proprietary Universal Protein Precipitating Agent (UPPA™). The UPPA agent cleans the protein samples from interfering agents prior to performing colorimetric reaction.

Reagent	Compatibility	Reagent	Compatibility
Amino Acids	-	NP-40	-
Ammonium Sulfate	40%	Octaethyleneglycol dodecyl ether	-
β-Mercaptoethanol	5%, 15%*	Octyl Glucoside	-
Brij® 35	1%	Phosphate Buffer	0.2M
Calcium Chloride	-	Sarcosyl	1%
CHAPS	4%	SDS	-
CHAPSO	1%	Sodium Azide	0.1M
CTAB	1M	Sodium Chloride	0.5M
Deoxycholate	-	Sodium Hydroxide	2.5M
Digitonin	-	Sucrose	30%
DTT	0.1M, 0.35M*	TCEP	15mM
EDTA	0.1M	Thesit®	2%
Glycerol	30%	Thiourea	2M
Guanidine.HCl	6M	Tributylphosphine (TBP)	-
Guanidine Thiocynate	6M	Tris (pH 8)	0.5M
HEPES	0.1M	Triton® X-100	3%
Hydrochloric Acid	-	Triton® X-114	3%
Imidazole	0.5M	Tween® 20	2%
Iodoacetamide	15mM	Urea	8M
Laemmli Buffer (w/5% β-Mercaptoethanol)	Compatible	Zwittergent® 3-12	1.5M

*Table 12: A selection of compounds and their maximum concentrations compatible with the CL Protein Assay. -, not tested; X, not compatible; *, two washes (optional).*

FEATURES

- Quantitation in the presence of detergents and reducing agents
- Quick 15 minute incubation
- Increased color stability (color will not change more than 5% in 1 hour or 10% in 2 hours)
- Linear response 0.2-1.5 mg/ml
- Convenient kit provides all necessary reagents for 250 standard (5ml) assays or 1000 microtube (1.5-2 ml) assays

APPLICATIONS

- For samples containing common laboratory agents, such as reducing agents, chelating agents (EDTA), and detergents.
- For samples containing chaotropic agents, drugs, antibiotics, cobalt, and numerous other agents and extraction buffers.

Cat. No.	Description	Size
786-1077	CL Protein Assay with BSA Standard	1000 assays
786-1078	CL Protein Assay with Non Animal Protein Standard	1000 assays
786-1084	CL Protein Assay with Pre-Diluted BSA Standard	1000 assays
786-1085	CL Protein Assay with Bovine γ Globulin Standard	1000 assays

Protein dotMETRIC™ Assay

1µl Assay For Rapid Protein Estimation

Rapidly, mix 1µl protein sample with the supplied Dilution Buffer and apply 1µl of the solution to the test strip by point-of-contact capillary action. Under the assay's specific buffer conditions the protein enters into the matrix of the test strip, binds and saturates as protein then diffuses in a circular manner. Develop the test strip in approximately 5 minutes. A circular protein spot is produced. The diameter of the spot is proportional to the concentration. By measuring the diameter of the spots with the Protein dotMETRIC™ scale you can easily determine concentration of protein. No expensive spectrophotometers or cuvettes required.

For increased reproducibility and test reliability, the dotMETRIC™ kits are supplied with an optional Spot Application Device. The Spot Application Device allows application of samples using fixed volume (1µl) capillary tips and simplifies the task of applying the protein solution on the test strips by point of contact capillary action. The Spot Application Device simplifies the application of one or more samples as well as it improves the reliability of results.

Gelatin, BSA, Avidin, alcohol dehydrogenase (yeast) and Thyroglobulin have been used to measure diameters of protein spots on the test strip at predetermined concentrations. It has been found that the diameters of protein spots on the test strip are not dependent on the nature and the origin of protein. Since the spot formation is not dependent on the amino acid composition of protein, this property makes the dotMETRIC™ assay independent of protein-to-protein variation.

The dotMETRIC™ assay is able to resist common laboratory agents such as Triton® X-100, Triton® X-114, Thesit®, Tween® 20, Nonidet® P-40, SDS, reducing agents such as ß-mercaptoethanol and DTT, sugars, cobalt, EDTA, Tris buffers, and so forth.

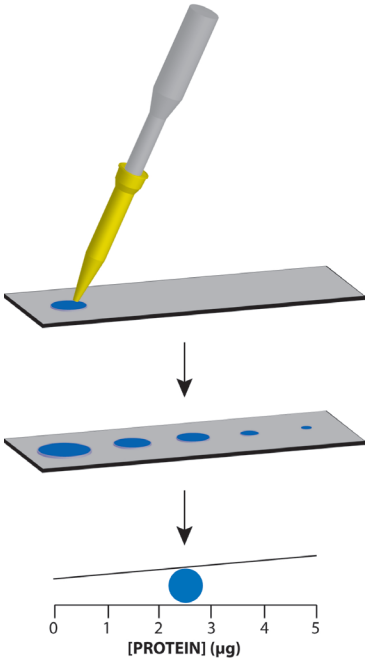

Figure 15: Protein dot METRIC™ Protein Assay Scheme.

When the protein spot diameters are below the measurability of the dotMETRIC™ scale, the assay employs a different strategy for protein concentration determination, known as the Dilution to the Limit of Detection (DLD™) Protocol.

According to the DLD™ Protocol, When a protein solution is serially diluted and spotted onto the test strip, a dilution is reached beyond which the protein spots are not visible; i.e., the dilution has reached the limit of detection (DLD™). This dilution factor is used to determine protein concentration.

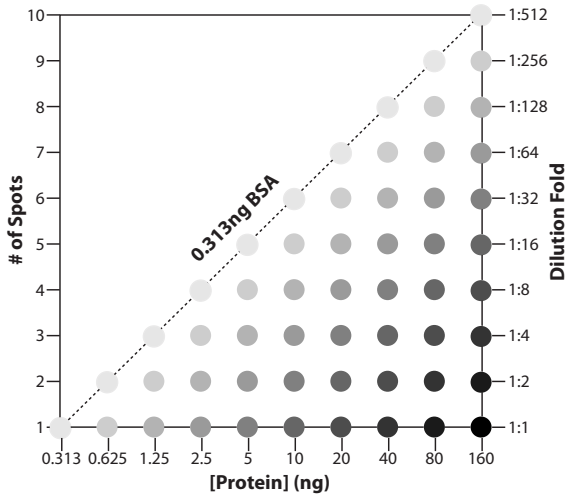

Figure 16: A representation of the DLD™ Protocol.

FEATURES

- **Sample Economy:** Use as little as 1µl of sample
- **Rapid Assay:** Takes 8-10 minutes and can assay as little as 2ng BSA
- **No Protein-to-Protein Variation:** Assay is independent of protein-to-protein variation
- Resistant to Detergents, Reducing Agents & Other Laboratory Agents

APPLICATIONS

- For rapid estimation of protein concentration
- For determination of protein concentration in cellular fractions, tissue & cell lysates and chromatography purification fractions
- For protein samples containing common laboratory agents, such as reducing agents (β-mercaptoethanol, DTT), chelating agents, detergents, amines, sugars and more
- To determine protein concentration in gel loading (SDS-PAGE) sample buffers
- When limited amount of sample is available for analysis; requires only 1µl protein sample

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More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-20	Protein dotMETRIC™ Kit	>300 Assays
786-21	Protein dotMETRIC™ Kit with Spot Application Device & Glass Capillary Tips	>300 Assays

Protein Assay Selection Guide

Assay Type	Interfering Agent Compatible	Interfering Agents Removed	Detergent Compatible (%)				Reducing Agent Compatible (M)		Protein-To-Protein Variation	Tubes Required For Assay	Sample Volume (µl)	Linear Response (µg)	Assay Time (mins)	Calibration Plot Required	Instrumentation Required
			SDS	Triton® X-100	Tween® 20	CHAPS	Mercaptoethanol	DTT							
CB-X™	Bradford/ Coomassie	****	Yes	2	2	2	2	1	1	Yes	One per sample	5-100	0.5-50	10	No	Spectrophotometer or plate reader; centrifuge
CB™	Bradford/ Coomassie	*	No	0.015	0.06	0.03	0.5	1	1	Yes	For Samples & Standards	10-100	0.5-50	30	Yes	Spectrophotometer or plate reader
DCB™	Bradford/ Coomassie	***	No	2	1	1	5	1	1	Yes	For Samples & Standards	50	0.5-50	15	Yes	Spectrophotometer or plate reader
FluoroRed 660	Fluorescent Dye Binding	*	No	0.1	0.1	0.1	NT	NT	0.01	Yes	For Samples & Standards	100	0.01-1	30	Yes	Fluorometer
RED 660™	Proprietary dye/metal complex	***	No	5	1	10	1	1	0.5	Yes	For Samples & Standards	10-50	0.5-20	<10	Yes	Spectrophotometer or plate reader
SPN™	Protein binding membrane	****	Yes	2	2	1	2	1	1	Yes	One per sample	1-10	0.5-10	10	No	Spectrophotometer or plate reader; centrifuge
SPN™-htp	Protein binding membrane	****	Yes	2	2	1	2	1	1	Yes	One per sample	1-10	0.5-10	10	No	Spectrophotometer or plate reader; centrifuge
NI™	Unbound Copper	****	Yes	1	3	2	1	0.32	0.1	Minimal	For Samples & Standards	1-50	0.5-50	30	Yes	Spectrophotometer; centrifuge
BCA	Bicinchoninic acid	**	No	5	5	5	5	0.001	0.001	Minimal	For Samples & Standards	50	1-100	30-120	Yes	Spectrophotometer or plate reader
Micro BCA	Bicinchoninic acid	**	No	5	5	5	5	0.001	0.001	Minimal	For Samples & Standards	1000	0.5-20	60-120	Yes	Spectrophotometer or plate reader
BCA Reducing Agent Compatible	Bicinchoninic acid	***	No	5	5	5	5	0.035	0.035	Minimal	For Samples & Standards	25	1-100	45-135	Yes	Spectrophotometer or plate reader

Assay Type	Interfering Agent Compatible	Interfering Agents Removed	Detergent Compatible (%)				Reducing Agent Compatible (M)		Protein-To-Protein Variation	Tubes Required For Assay	Sample Volume (µl)	Linear Response (µg)	Assay Time (mins)	Calibration Plot Required	Instrumentation Required
			SDS	Triton® X-100	Tween® 20	CHAPS	Mercaptoethanol	DTT							
ML (Modified Lowry)	Copper Binding	**	No	10	1	1	1	-	0.001	Minimal	For Samples & Standards	5-100	0.5-50	20	Yes	Spectrophotometer or plate reader
CL- (Compatible Lowry)	Copper Binding	****	Yes	2	3	2	4	5-15	0.1-0.35	Minimal	For Samples & Standards	25-100	0.5-50	40	Yes	Spectrophotometer or plate reader, centrifuge
dotMETRIC™	Test strip	****	Yes	2	2	2	2	1	1	Minimal	No	1	0.025-1	10	No	None

Table 13: Selection Guide for Protein Estimation Assays.

ACCESSORIES FOR PROTEIN dotMETRIC™

Spot Application Device

For use with Protein dotMETRIC™ protein assay. Simplifies the application of one or more samples and improves reliability of results.

Application Glass Capillary Tips

1µl Application glass capillary tips for use with Spot Application device in the Protein dotMETRIC™ Protein assay. Simplifies the application of one or more samples as well as improves reliability of results.

Sample Application (pipette) Tips

For use with Protein dotMETRIC™ protein assay, 1-10µl pipettor tips to be used with standard laboratory pipettes.

Developing Trays

Trays for developing test strips for Protein dotMETRIC™ protein assay.

Cat. No.	Description	Size
786-63	dotMETRIC™ Spot Application Device	1
786-23	1µl Application Glass Capillary Tips	100
786-64	Sample Application (pipette) Tips	96 tips
786-24	Developing Trays	2

ACCESSORIES FOR PROTEIN ASSAYS

For researchers' convenience, G-Biosciences offers a wide selection of accessories and supplies for protein assays.

Bovine Serum Albumin Standard

BSA standard (2mg/ml) prepared in saline buffer. Standard is supplied as 2 x 5ml aliquot.

Prediluted BSA Protein Standards

BSA protein standards in an easy-to-use prediluted format. Supplied in 6 x 5ml aliquots ranging from 0.1mg/ml-1.0mg/ml (0.1, 0.2, 0.3, 0.5, 0.8 and 1mg/ml).

Bovine γ-Globulin Protein Standards

γ-globulin standard (2mg/ml) prepared in saline buffer. The standard is supplied as 2 x 5ml or 10 x 5ml aliquots.

Prediluted Bovine γ-Globulin Protein Standards

γ-Globulin protein standards in an easy-to-use prediluted format. The Prediluted Protein Standards are supplied in 6 x 5ml aliquots ranging from 0.1mg/ml-1.0mg/ml.

Non Animal Protein Standard

Non Animal Protein Standards are protein standards for use in protein estimations that are generated from plant proteins. Standard is supplied as 2 x 5ml aliquot at a concentration of 2mg/ml.

Prediluted Non Animal Protein Standards

Ready to use solutions prepared in an aqueous buffer with a preservative for product stability. They are provided in six different concentrations ranging from 0.1mg/ml to 1.0mg/ml (0.1, 0.2, 0.3, 0.5, 0.8 and 1mg/ml).

Assay Tubes

Protein assay tubes, 2ml reaction volumes. For proper mixing and good color development.

Assay Cuvettes

Spectrophotometer assay cuvettes, 1ml reaction capacity, 500 cuvettes per box.

Cat. No.	Description	Size
786-006	Bovine Serum Albumin Standard (2 mg/ml)	2 x 5ml
786-744	Bovine Serum Albumin Standard (2 mg/ml)	1L
786-920	Bovine Serum Albumin Standard (2 mg/ml)	100ml
786-114	Prediluted Bovine Serum Albumin Standard	6 x 5ml
786-007	Bovine γ-Globulin Protein Standard (2 mg/ml)	2 x 5ml
786-010	Bovine γ-Globulin Protein Standard (2 mg/ml)	10 x 15ml
786-114G	Prediluted Bovine γ-Globulin Protein Standard	6 x 5ml
786-438	Non Animal Protein Standards [2mg/ml]	2 x 5ml
786-439	Prediluted Non Animal Protein Standards	6 x 5ml
786-008	Assay Tubes (2ml)	500
786-009	Assay Cuvettes (1ml)	500
786-009A	Assay Cuvettes (1ml)	100

Bradford Protein Assay: Calculation of an Unknown Standard

The traditional method for calculating protein concentration of an unknown sample is to use a standard curve that is generated from known protein standards.

The most reliable protein estimation is performed using a reference or a protein standard that has properties similar to the protein being estimated. Often, it is difficult to find a protein standard with similar properties to the sample being analyzed. As a result, it has become acceptable to use readily available proteins such as bovine serum albumin (BSA) and gamma globulin as standards. Using either the BSA or the bovine γ-globulin (IgG) as reference proteins, Bradford protein assays do show significant protein-to-protein variation; hence, the calculated result is an estimation of protein concentration.

A key point to remember is that identically assayed samples are directly comparable. This means that unknown samples and standards that are treated identically are directly comparable in terms of protein estimation. As a result, it is highly recommended to use the same buffers that your unknown samples are in for the generation of your standards.

How to generate protein standards?

Below is a simple table for the generation of your standards. As a general rule of thumb, use at least 6 standards for generating the standard curve and adjust the dilutions of standards to cover the expected range of your unknown samples. More importantly, stay within the linear range of your protein assay.

The buffer of choice should be the same buffer your unknown protein standards are prepared that way you are comparing like to like (apples to apples as opposed to apples and oranges).

Always include a blank of just the Buffer of Choice!

Tube #	Standard (µl)	Buffer of Choice (µl)	Final Concentration (mg/ml)	Final Volume (µl)
1	500µl of Starting 2mg/ml standard	0	2	500
2	750µl of Starting 2mg/ml standard	250	1.5	750
3	500µl of Starting 2mg/ml standard	500	1	500
4	250µl of Tube #2 Standard	250	0.75	500
5	500µl of Tube #3 Standard	500	0.5	500
6	500µl of Tube #5 Standard	500	0.25	500
7	500µl of Tube #6 Standard	500	0.125	750
8	-	500	0	500

Alternatively, you can purchase pre-diluted protein standards.

What are the units for my protein concentration?

The simple adage is: **"Units in =Units Out"**

In the table above, the units of the protein standards is mg/ml, which is the same as µg/µl, so your unknown standards concentration will be defined as mg/ml or µg/µl. In some cases, the standard curves are displayed with just µg of protein. G-Biosciences' Bradford Assay, CB™ Protein Assay, uses 50µl of protein standard. So, the µg of protein for the standards would be:

Volume of Protein Standard (ml) x Starting Protein Concentration= Amount of protein (mg)

0.05 x 2=0.1mg or 100µg

Tube #	Final Concentration (mg/ml)	Amount of Protein (µg)
1	2	100
2	1.5	75
3	1	50
4	0.75	37.5
5	0.5	25
6	0.25	12.5
7	0.125	6.25
8	0	0

The above table is shown to help lessen the confusion when standard curves or protein assays are performed based on the amount of protein; however, most researchers want to know the amount of protein in their sample, not the amount of protein in the assay tube or well. So, it is critical to always generate standard curves based on their starting concentrations.

How to Apply Dilution Factors

Most commercial assays will express the linear range of the assay, the range over which the assay is accurate. You should ensure that your unknown sample is within that linear range. If you know your sample is greater than the linear range, or if after performing an assay is outside the linear range, then you would need to dilute the sample.

For example, if the unknown sample is expected to have a concentration of 5mg/ml and the linear range of your assay is 0.1-1mg/ml, then the unknown sample needs to be diluted 10 fold so it is in the middle of the linear range. A 10-fold dilution would be 1 part unknown sample to 9 parts buffer of choice, or 100µl unknown sample added to 900µl buffer of choice.

Perform the assay and calculate the standard (see below). The result should be around 0.5mg/ml. To calculate the concentration of the undiluted, unknown sample, simply multiply by the dilution factor. So, 0.5 x 10= 5mg/ml.

Protein Standard Curve or Linear Plot

A common question is should you use a linear plot or a curve (a curvilinear regression). The two images below show the variations seen with a linear plot and a 3 parameter polynomial equation.

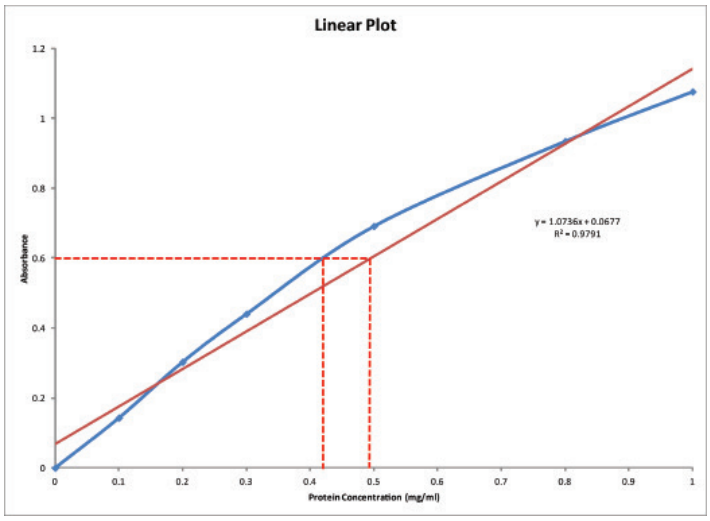


Figure 17: Linear plot.

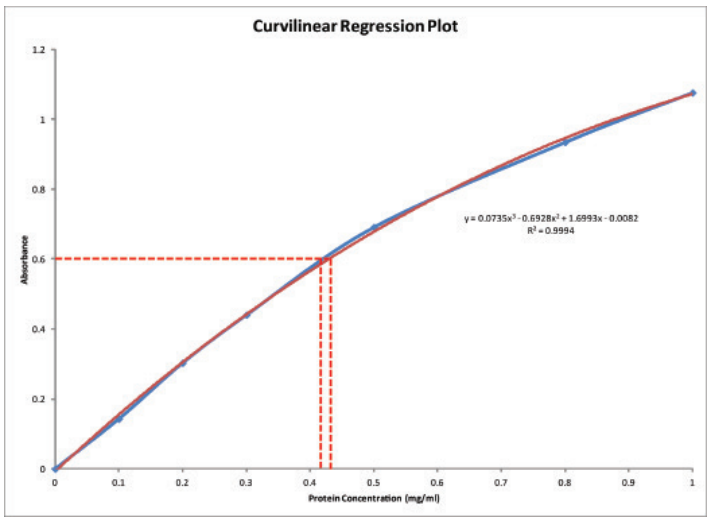


Figure 18: Curvilinear plot.

The blue lines show the purely graphical basis of a point to point curve and for an unknown sample with absorbance of 0.6 gives a protein estimation of ~0.42mg/ml.

Many researchers, for convenience, plot a linear regression for the set of standards as they assume the overall relationship between the standards is best described by a linear relationship (R2=0.9791), which is rarely seen. The red line in the Linear Plot figure shows the linear plot and the equation for this line is y=1.0736x + 0.0677. Solving for x (the protein concentration) for an absorbance of 0.6 gives:

x=(y-0.0677)/1.0736, so x=0.495mg/ml

As you can see by the Linear plot number above, a linear regression does not provide a good method for comparing the protein standards to the unknown samples; however it is a convenient method, providing a rough estimation.

For a more accurate estimation, and a feature of many new spectrophotometers and plate readers, is to use a curvilinear regression. The equation of the above curve is:

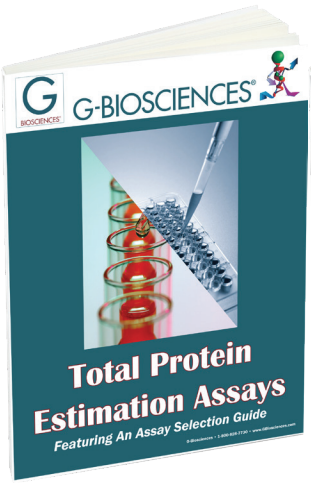
$y = 0.0735x^3 - 0.6928x^2 + 1.6993x - 0.0082$ and to solve for x is 0.425mg/ml, a more accurate estimation.

Helpful Hint

If you do not have the spectrophotometers or plate readers that can do the above calculations for you then here's how to use Microsoft Excel.

1. In column A place the Known protein concentrations of the standards
2. In column B, add the corresponding absorbances
3. Select both columns and from the insert menu select an XY Scatter chart.
4. Click on the corresponding chart and add a trendline. Choose polynomial and set the order to 2, 3 or 4 to achieve the best fit.
5. Make sure to select Display equation on the chart.
6. Use the resulting equation to calculate the protein concentration (x) from know absorbances.

Standard curves are always plotted as the Standards on the x-axis and absorbances on the y-axis, however to make solving the polynomial equations easier, plot absorbances on the x-axis and protein concentration on the y-axis. Now simply plug the absorbances (x) into the polynomial equation and solve for y (Protein concentration).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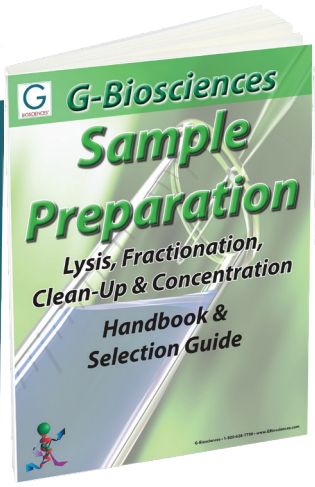
Total Protein Estimation Assays
Featuring An Assay Selection Guide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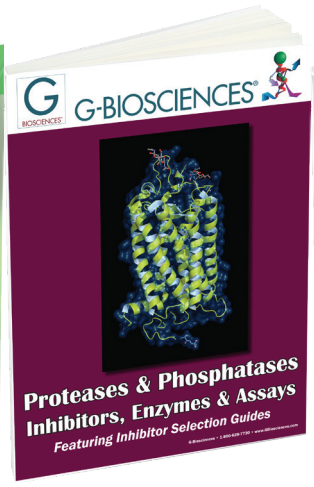
Lysis, Fractionation, Clean-Up & Concentration
Handbook & Selection Guide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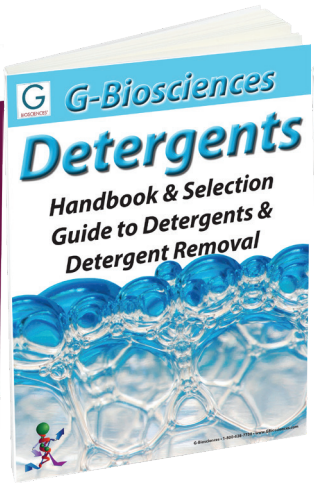
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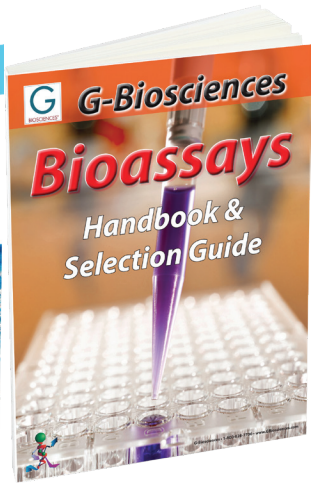
Handbook & Selection Guide to Detergents & Detergent Removal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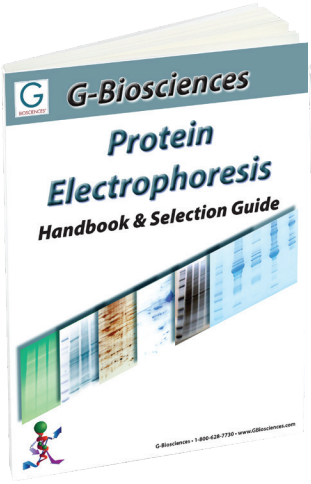
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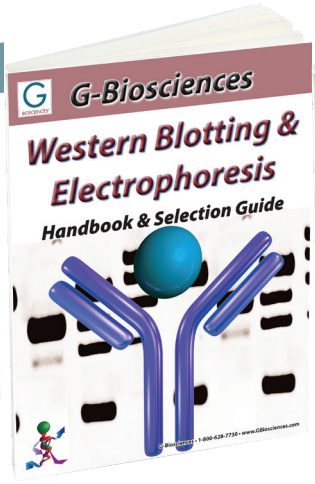
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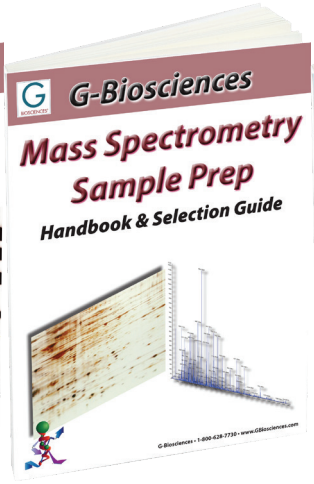
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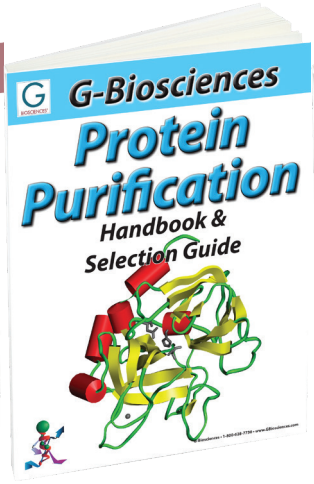
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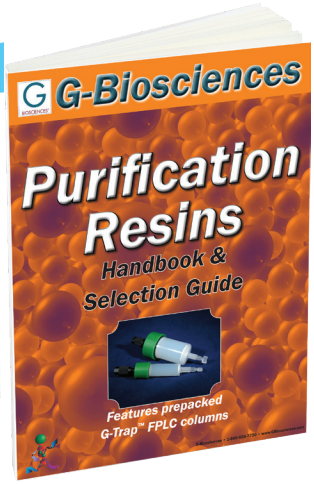
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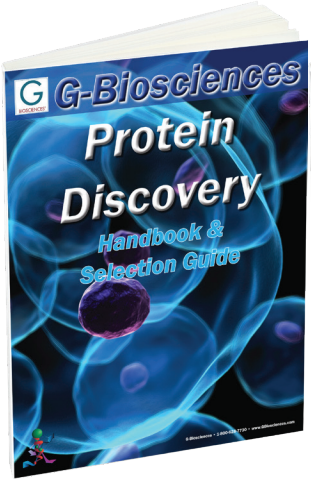
Handbook & Selection Guide
Features prepacked G-Trap™ FPLC columns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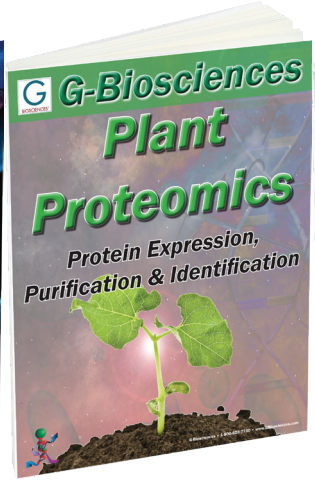
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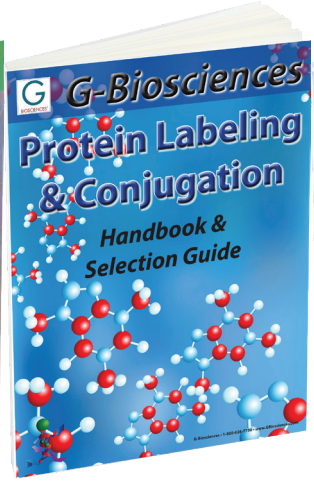
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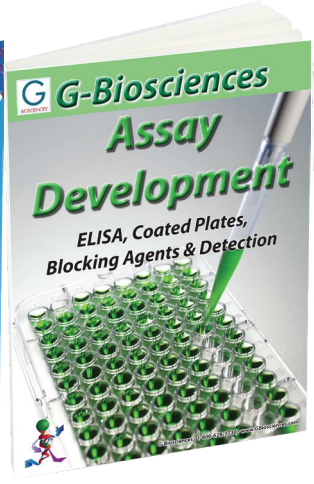
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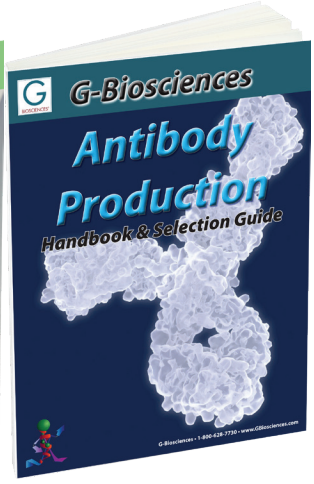
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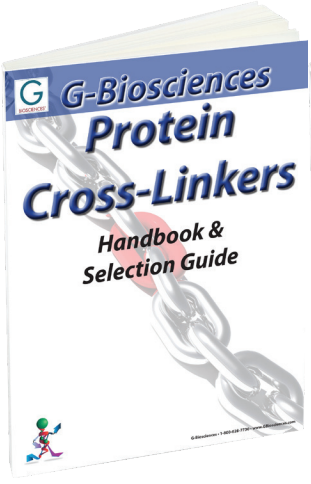
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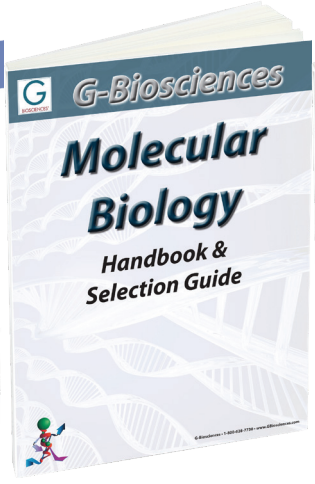
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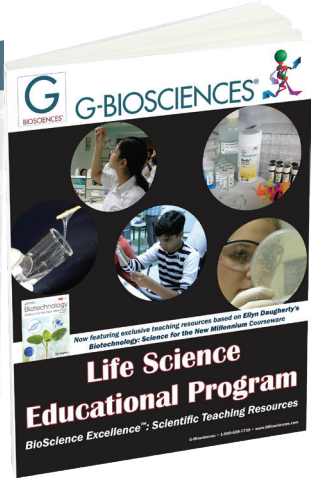
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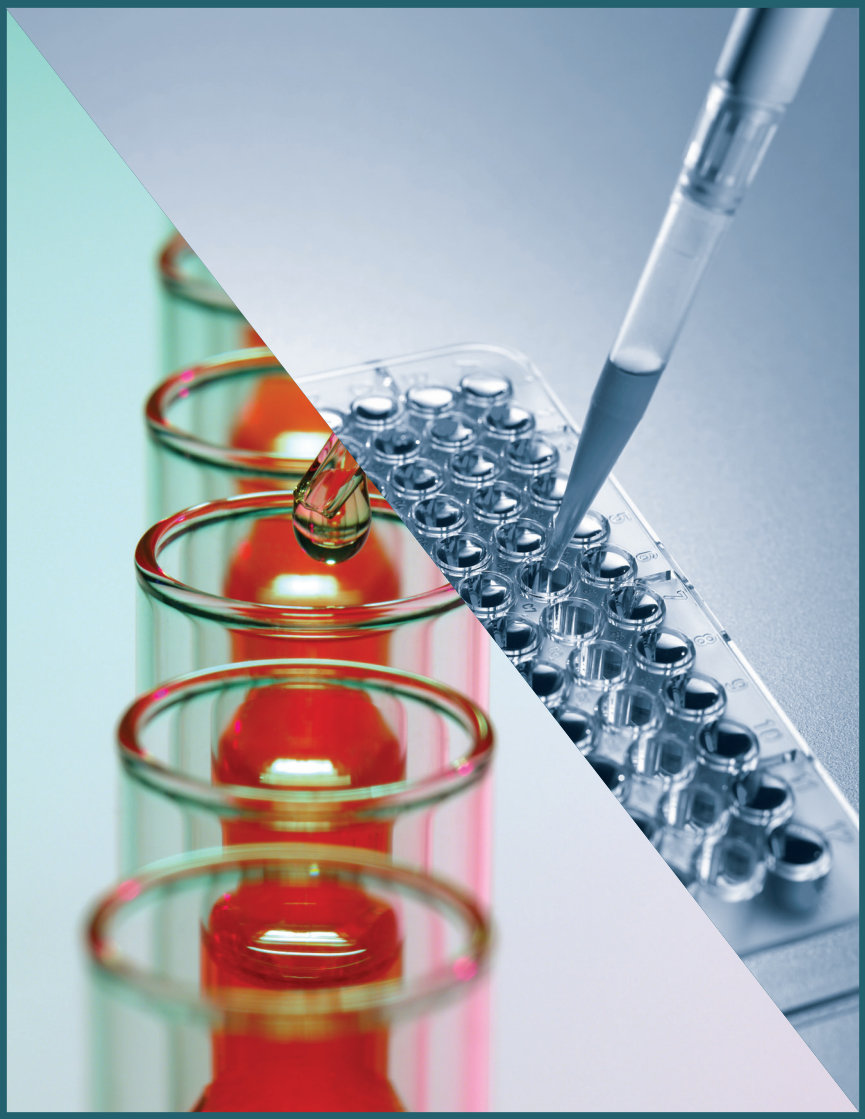

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