

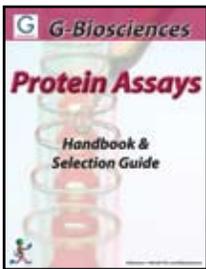


G-Biosciences

Protein Assays

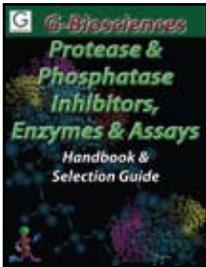
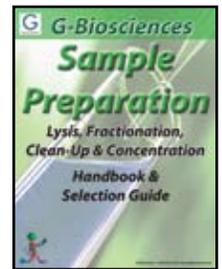
Handbook & Selection Guide





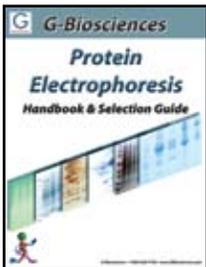
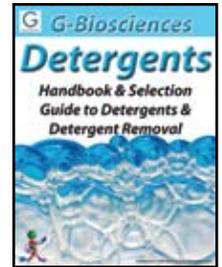
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- **Electrophoresis Clean-Up**
- **Concentration Systems**
- **Contamination Removal**



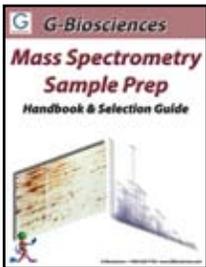
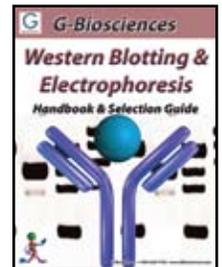
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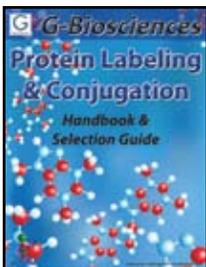
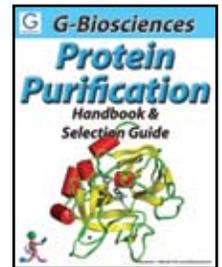
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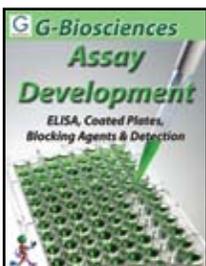
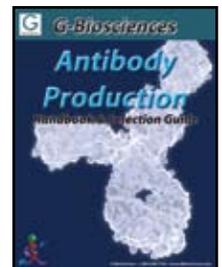
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- **Buffers & Reagents**



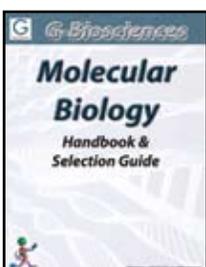
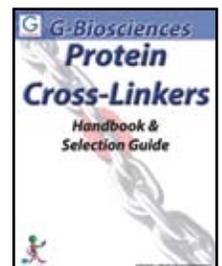
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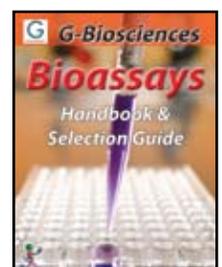
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Introduction

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.

GBiosciences 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 ($\lambda_{max}=465\text{nm}$) to blue ($\lambda_{max}=610\text{nm}$). 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™ and CB-X™ protein assays are dye binding protein assays.

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.

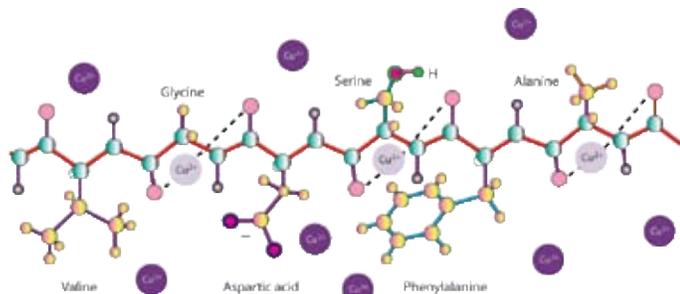


Figure 1: The interaction of copper ions with proteins.

The presence of tyrosine, tryptophan, cysteine, histidine and asparagine 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.

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 strips and spot application for protein estimation.

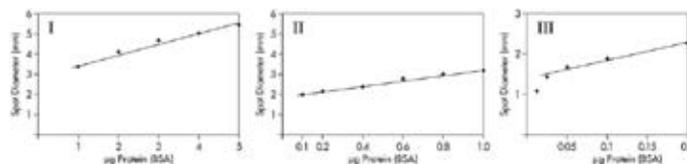


Figure 2: The linear relationship of protein BSA concentration with the protein spot diameters.

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 effects of non-protein agents present in the protein samples.

Non-Interfering™ (NI™) Protein Assays, CB-X™, SPN™ and SPN™-htp 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.

The dotMETRIC™ protein assay on the other hand is designed to circumvent the interfering effects of non-protein agents in the protein solution.

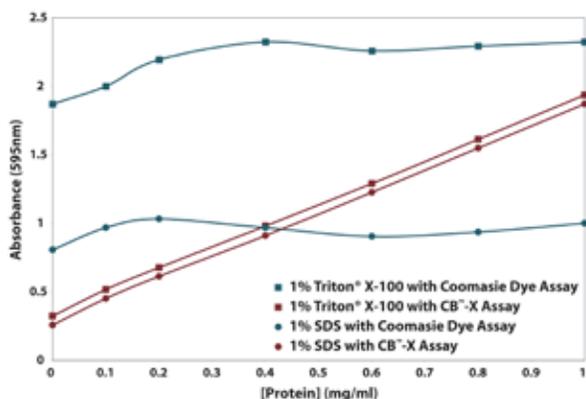


Figure 3: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A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

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:

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 0.5 μ g 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 1 μ g 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 0.5 μ g 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.

Protein Estimation Assays

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 interfering 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 an 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.

A set of bovine serum albumin standards are supplied for generating curves when using CB-X™ Assay Dye alone or for researchers who prefer to generate their own standard curve or to generate their own CB-X™ Table for 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.

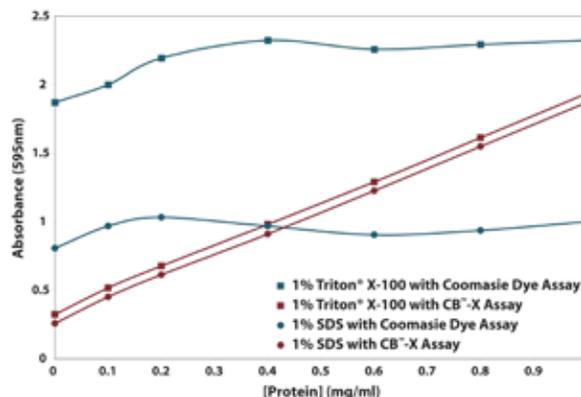


Figure 4: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A 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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Cat. #	Description	Size
786-12X	CB-X™ Protein Assay with Albumin Standard	500 Assays
786-12XT	CB-X™ Protein Assay Trial	10 Assays

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.

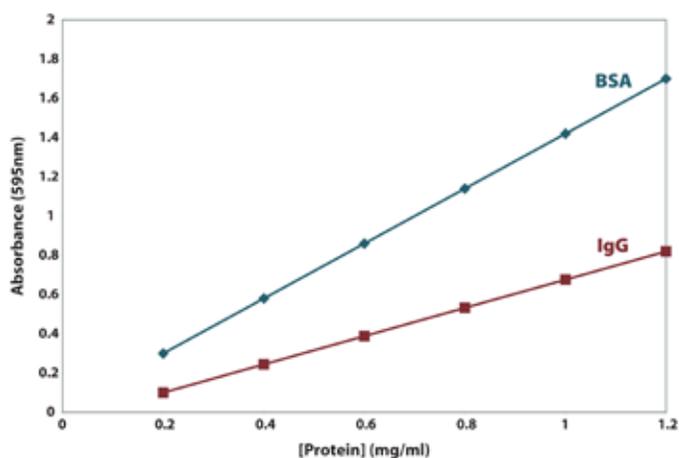


Figure 5: Linear Response and Protein-to-Protein variation with CB™ Protein Assay.

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. Table 2 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.

REFERENCES

Bradford, M. M. (1976), *Anal. Biochem.* 72:248

Cat. #	Description	Size
786-012	CB™ Protein Assay with Albumin Standard	500 Assays

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

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

Protein Estimation Assays

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

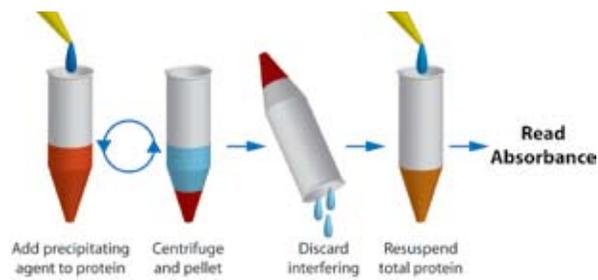


Figure 6: The NI™ Protein Assay Scheme

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.

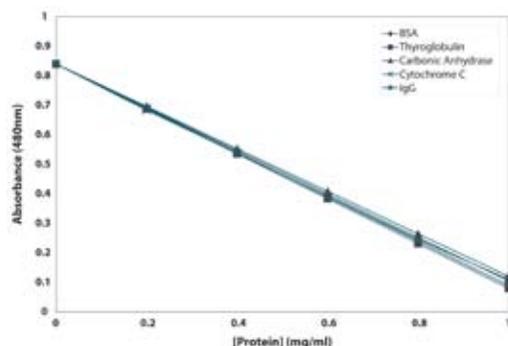


Figure 7: NI™ Protein Assay shows minimal protein-to-protein variation. BSA, thyroglobulin, carbonic anhydrase, cytochrome C and bovine immunoglobulin G were assayed for protein-to-protein variation. The proteins produced identical color and slope in the range of 0.5µg-50µg, giving an average ratio of 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 3: 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 4: A selection of compounds and the maximum concentrations that are compatible with the NI™ Protein Assay.

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Cat. #	Description	Size
786-005	NI™ (Non-Interfering™) Protein Assay Kit with Albumin Standard	500 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 further 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 5: Protein-to-protein variation.

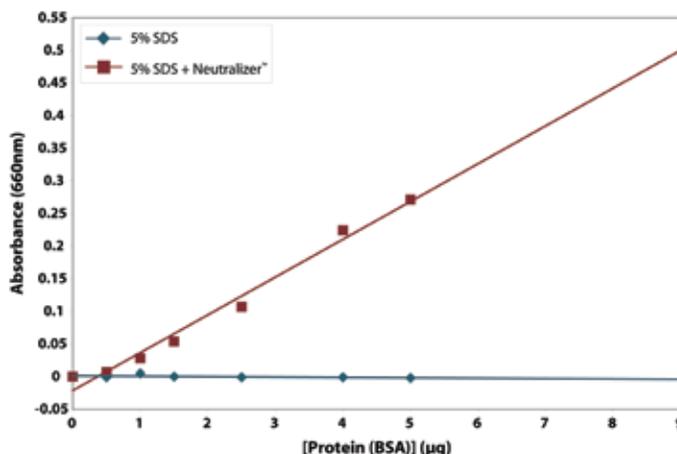


Figure 8: Presence of Neutralizer™ overcomes 5% SDS interference.

Cat. #	Description	Size
786-676	RED 660™ Protein Assay	500 standard/ 2,500 microwell
786-603	Neutralizer™	10 vials
786-604	Neutralizer™	12.5g
786-673	Neutralizer™	5 vials

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%	Octylthioglycopyranoside	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 6: A selection of compounds and buffers with their maximum concentrations that are compatible with the RED 660™ Protein Assay.

Protein Estimation Assays

Bicinchoninic Acid (BCA) 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 7: 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

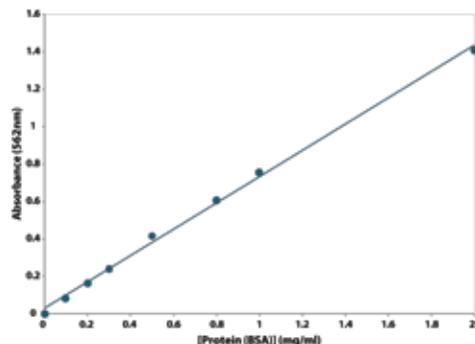


Figure 9: Bicinchoninic Acid (BCA) Protein Assay Standard Curve.

Micro Bicinchoninic Acid (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 (BCA) 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 β-mercaptoethanol.

The Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay is suitable for quantifying protein solutions in 1ml assays. This kit is suitable for is 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 8: Compatible substances for Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay.

Cat. #	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
786-572	Micro Bicinchoninic Acid (BCA) Protein Assay	500 x 1ml assays >3,300 x microwell assays
786-573	Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay	250 x 1ml assays

SPN™ Protein Assay

An Ultra Sensitive Spin Format Protein Assay!

A novel protein assay that is suitable for 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. (Patents pending)

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 10: 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-automated 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 11: The SPN™-htp protein assay.

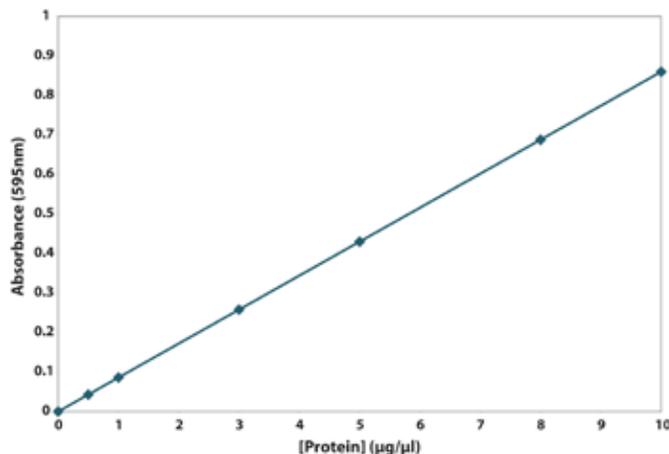


Figure 12: Standard Calibration Plot for SPN™ Protein Assay.

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

REFERENCES

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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 9: Compatible substances for SPN™ Protein Assay.

Cat. #	Description	Size
786-020	SPN™ Protein Assay Kit	50 Assays
786-021	SPN™-htp Protein Assay Kit	5 x 96 assay plates

Protein Estimation 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 β-mercaptoethanol and DTT, sugars, cobalt, EDTA, Tris buffers, and so forth.

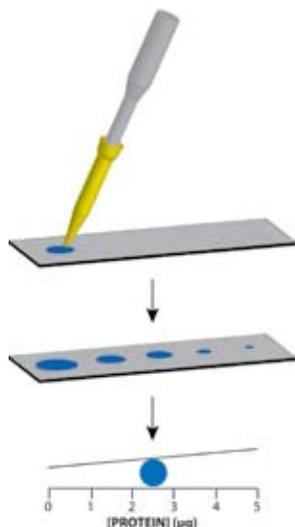


Figure 13: Protein dot METRIC™ Protein Assay Scheme.

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 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.

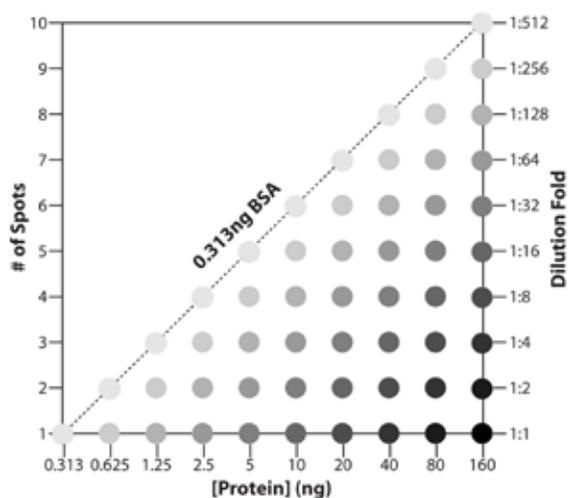


Figure 14: A representation of the DLD™ Protocol.

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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Muresan, V. et al (1996) *J. Cell Biol.* 135: 383

Cat. #	Description	Size
786-20	Protein dotMETRIC™ Kit	>300 Assays
786-21	Protein dotMETRIC™ Kit with Spot Application Device & Glass Capillary Tips	>300 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. #	Description	Size
786-006	Bovine Serum Albumin Standard (2mg/ml)	2 x 5ml
786-114	Prediluted Bovine Serum Albumin Standard (0.1-1.0mg/ml)	6 x 5ml
786-007	Bovine γ-Globulin Protein Standard (2mg/ml)	2 x 5ml
786-010	Bovine γ-Globulin Protein Standard (2mg/ml)	10 x 15ml
786-114G	Prediluted Bovine γ-Globulin Protein Standard (0.1-1.0mg/ml)	6 x 5ml
786-008	Assay Tubes (2ml)	500
786-009	Assay Cuvettes (1ml)	500
786-009A	Assay Cuvettes (1ml)	100
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.

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.

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™ (786-12X)	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™ (786-012)	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
NI™ (786-005)	Unbound Copper	****	Yes	1	3	2	1	0.32	0.1	Minimal	For Samples & Standards	1-50	0.5-50	30	Yes	Spectrophotometer, centrifuge
RED 660™ (786-676)	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
BCA (786-570/ 571)	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 (786-572)	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 (786-573)	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
SPN™ (786-020)	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 (786-021)	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
dotMETRIC™ (786-20)	Test strip	****	Yes	2	2	2	2	1	1	Minimal	No	1	0.025-1	10	No	None

Table 10: Selection Guide for Protein Estimation Assays.

ProteSEEKER™

Identify Destructive Proteases

ProteSEEKER™ identifies specific types of proteases with a panel of twelve protease inhibitors and a sensitive colorimetric protease assay.

ProteSEEKER™ allows researchers to screen their protein samples and establish which specific class of proteases are present and therefore design a highly specific protease inhibitor cocktail using the minimal number of protease inhibitors. Alternatively, ProteSEEKER™ can be used to test existing protease inhibitor cocktails and identify their inadequacies and therefore supplement in additional protease inhibitors.

ProteSEEKER™ protease screening assay consists of a ready-to-use dye-labeled protein, which is digested by proteases to release dye-labeled peptides. The absorbance of which is measured for determination of protease activity. The inhibitors are supplied at a 100X concentration and the 1X concentration provides >90% inhibition in most biological samples.

Protease Screening Kit

The Protease Screening Kit provides you with a simple and quick method for testing your samples for proteolysis. Simply incubate your sample in the reagent provided and obtain results. The kit uses dye-labeled protein conjugate as protease substrate, which allows nanogram level detection. The absorbance of dye-labeled peptide is measured at 574nm for determination of protease activity. The kit is sufficient for 50 assays in a micro well format.

APPLICATIONS

- Screening samples for protease activity

REFERENCES

Person, M.D. et al (2006) *J. Biomol. Tech.* 17: 145
Razeghi, P. et al (2007) *Mol. Cell Cardiol.* 42: 449

Protease Assay Kit

The Protease Assay Kit is designed for the quantitative determination of proteases present in a protein sample, using a dye-labeled substrate.

The proteases present in the sample of interest will digest the protein substrate and release dye labeled peptides. The absorbance of the dye-labeled peptide is measured at 570nm for determination of protease activity.

Chemically stabilized Trypsin (MSG-Trypsin™) is supplied with the kit as a general protease standard; however, other specific protease standards can also be used. MSG-Trypsin™ is an ultra-pure trypsin from bovine pancreas, modified by methylation followed by TPCK treatment and is resistant to autolysis.

The kit components are sufficient for 50 assays in a microtiter plate format or 0.5ml assay tubes.

APPLICATIONS

- Determination of protease activity in biological samples, with nanogram detection levels

Fluoro™ Protease Assay

Fluorometric, Quantitative Protease Assay

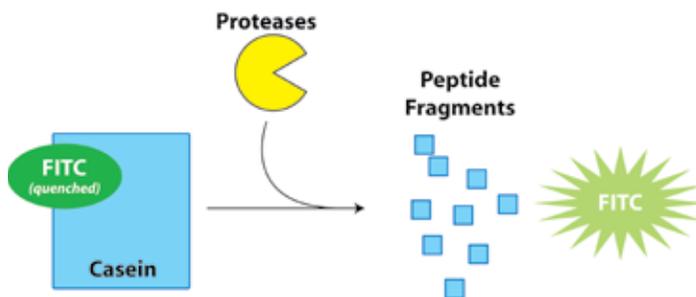


Figure 15: Fluoro™ Protease Assay Scheme

The Fluoro™ Protease Assay Kit is designed for the quantitative determination of proteases present in a protein sample. The assay uses fluorescein isothiocyanate (FITC)-labeled casein as a general protease substrate. The fluorescein label on the FITC-casein is highly quenched. When the proteases present in the sample of interest digest the FITC-casein substrate into smaller peptides, the quenching of the fluorescence label is relieved and the fluorescence of the substrate is increased. The fluorescence of the FITC-labeled peptide is measured with excitation at 485nm and emission at 535nm to determine protease activity. The kit detects picogram level of proteases present in the sample.

The kit is supplied with our chemically stabilized MSG-Trypsin™ for use as a general protease control; however, other specific protease standard controls can be used. MSG-Trypsin™ is an ultra-pure trypsin from porcine pancreas, modified by methylation followed by TPCK treatment and is extremely resistant to autolysis. The kit components are sufficient for 1,000 assays in a microtiter plate format.

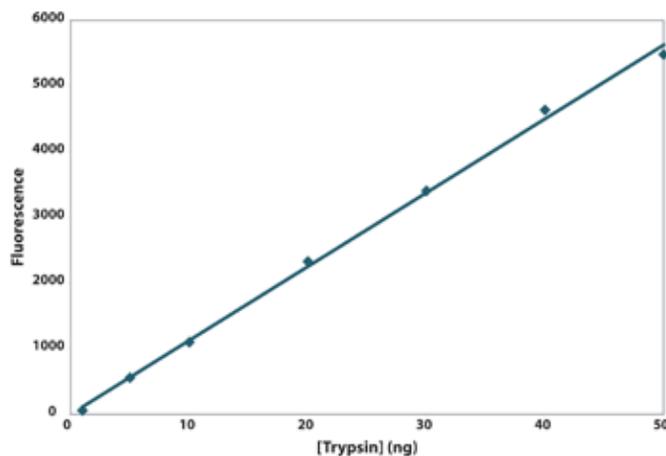


Figure 16: The Linear Response of Fluoro™ Protease Assay

APPLICATIONS

- Quantitative fluorescence protease assay

Cat. #	Description	Size
786-325	ProteSEEKER™	50 Assays
786-137	Protease Screening Kit	50 Assays
786-028	Protease Assay Kit	50 Assays
786-320	Fluoro™ Protease Assay Kit	1000 Assays

Phosphatase Assays

Phosphatase Assay

A pNPP based assay for simple phosphatase estimation

The Phosphatase Assay kit is designed to measure the activity of phosphatases in biological samples and to screen for agonists and inhibitors of phosphatases.

The Phosphatase Assay kit uses para-nitrophenyl phosphate (pNPP), a chromogenic substrate for most phosphatases, including alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases.

The phosphatases remove the phosphate group to generate p-nitrophenol, which is deprotonated under alkaline conditions to produce p-nitrophenolate that has strong absorption at 405nm.

The kit components are sufficient for performing up to 1000 assays in 96-well plate format and easily adaptable to cuvettes or 384-well plates.

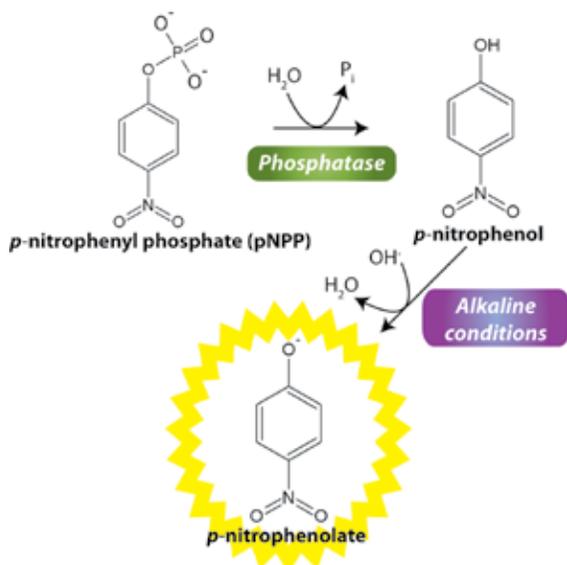


Figure 17: The Phosphatase Assay Scheme

FEATURES

- A colorimetric, pNPP based assay
- Measure phosphatase activity in biological samples
- Screen for phosphatase agonists and inhibitors

APPLICATIONS

- For the quantification of phosphatase activity
- To screen for agonists and inhibitors of phosphatases

Cat. #	Description	Size
786-453	Phosphatase Assay Kit	1000 Assays

PhosphoQuant™

Estimation of phosphates in phosphoproteins

PhosphoQuant™ is specifically designed for quick and reliable determination of whether a purified protein is phosphorylated and the extent of phosphorylation. The assay is based on the alkaline hydrolysis of phosphates from seryl and threonyl residues in phosphoproteins and the subsequent quantification of the released phosphate with a Molybdate dye.

Cat. #	Description	Size
786-256	PhosphoQuant™	400 assays

PHOSPHATASE INHIBITOR COCKTAILS

The PhosphataseArrest™ phosphatase inhibitor cocktails are ready-to-use 100X solutions that are simply added to your extraction buffers or samples.

FEATURES

- Single 100X solution
- Ready-to-use
- Compatible with most phosphatase assays
- No resuspension required

PhosphataseArrest™ I

A broad spectrum phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target serine/threonine specific, tyrosine specific and dual specificity phosphatases.

PhosphataseArrest™ I is a stabilized solution of sodium fluoride, sodium orthovanadate, sodium pyrophosphate, β-glycerophosphate & sodium molybdate.

REFERENCES

Siegel, D. et al (2011) *J. Pharmacol. Exp. Ther.* 336:874-880
Garrido-Lecca, A. and Blumenthal, T. (2010) *Mol. Cell. Biol.* 30:3887-3893

PhosphataseArrest™ II

A phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target acid, alkaline and tyrosine phosphatases.

PhosphataseArrest™ II contains optimized concentrations of sodium fluoride, sodium tartrate, sodium orthovanadate, imidazole & sodium molybdate.

PhosphataseArrest™ III

A phosphatase inhibitor cocktail consisting of three phosphatase inhibitors that target alkaline and serine/threonine phosphatases.

PhosphataseArrest™ III is a stable, convenient 100X solution of cantharidin, bromotetramisole, and microcystin LR.

PhosphataseArrest™ IV

A phosphatase inhibitor cocktail consisting of three phosphatase inhibitors, that target alkaline and serine/threonine phosphatases.

PhosphataseArrest™ IV is a stable, convenient solution of cantharidin, p-bromotetramisole oxalate and calyculin.

Cat. #	Description	Size
786-450	PhosphataseArrest™ I [100X]	1ml
786-451	PhosphataseArrest™ II [100X]	1ml
786-452	PhosphataseArrest™ III [100X]	1ml
786-602	PhosphataseArrest™ IV [100X]	1ml

CasPASE™ Apoptosis Assays

Sensitive fluorometric and colorimetric assays in one

The CasPASE™ Apoptosis assays are designed to monitor apoptosis by measuring caspase activity, an early indicator of apoptosis. The assay can be monitored with a fluorescence reader, spectrophotometer or a titer plate reader.

The kit uses AFC-substrate (7-amino-4-trifluoromethyl coumarin conjugated at C-terminal), which exhibits both fluorescence and absorbance spectral shifts between the substrate-conjugate and the free dye (AFC). The AFC substrate is both chromogenic (yellow-green color visible to the naked eyes) and fluorogenic (detected at 510-550nm with a fluorometer). During the reaction, the substrate releases AFC free dye and undergoes both a fluorescence and absorbance shift. Since AFC-substrate exhibits dual-mode detection capability, arbitrary fluorescence values can be standardized to an optical density value.

When measuring fluorescence, the assay reaction is excited at 360-390nm and emission is read at 510-550nm. If the assay is measured with a spectrophotometer or a titer plate reader, the absorbance is measured at 360-390nm.

The assay can be conveniently adapted for high-throughput 96-well format. The assay system may be used with purified enzyme preparations, cell extracts or tissue lysates.

CasPASE™ assays are available for caspase enzymes 1-10 and 13. Each CasPASE™ assay kit is supplied with necessary assay buffers, enzyme specific AFC-substrate, free dye (AFC) and the potent caspase inhibitor Z-VAD-FMK for establishing proper positive and negative controls and standards. Available in a 50 or 100 assay size.

FEATURES

- Assays for caspase enzymes 1-10 and 13 available
- Both fluorescence & absorbance spectral shifts
- All kits supplied with enzyme specific AFC-substrate, free dye (AFC) & inhibitor Z-VAD-FMK

Cat. #	Description	Size
786-200A	CasPASE™ 1, 4, 5 assay with Ac-WEHD-AFC	50 assays
786-200B	CasPASE™ 1, 4, 5 assay with Ac-WEHD-AFC	100 assays
786-201A	CasPASE™ 2 assay with Ac-VDVAD-AFC	50 assays
786-201B	CasPASE™ 2 assay with Ac-VDVAD-AFC	100 assays
786-202A	CasPASE™ 3, 7, 10 assay with Ac-DEVD-AFC	50 assays
786-202B	CasPASE™ 3, 7, 10 assay with Ac-DEVD-AFC	100 assays
786-203A	CasPASE™ 6 assay with Ac-VEID-AFC	50 assays
786-203B	CasPASE™ 6 assay with Ac-VEID-AFC	100 assays
786-204A	CasPASE™ 8 assay with Ac-LETD-AFC	50 assays
786-204B	CasPASE™ 8 assay with Ac-LETD-AFC	100 assays
786-205A	CasPASE™ 9 assay with Ac-LEHD-AFC	50 assays
786-205B	CasPASE™ 9 assay with Ac-LEHD-AFC	100 assays
786-206A	CasPASE™ 13 assay with Ac-LEED-AFC	50 assays
786-206B	CasPASE™ 13 assay with Ac-LEED-AFC	100 assays

Recombinant Human Caspases

Active caspases for screening caspase inhibitors

Recombinant human caspases 1-3 and 6-10 are supplied lyophilized in quantities of 25 units. One unit of the specific recombinant caspase is the enzyme activity that cleaves 1nmol of a specific caspase substrate per hour at 37°C in a reaction solution containing 50mM HEPES (pH7.2), 50mM NaCl, 0.1% CHAPS, 10mM EDTA, 5% Glycerol and 10mM DTT.

Cat. #	Description	Size
RCH-001	Recombinant Human Caspase 1	25 units
RCH-002	Recombinant Human Caspase 2	25 units
RCH-003	Recombinant Human Caspase 3	25 units
RCH-006	Recombinant Human Caspase 6	25 units
RCH-007	Recombinant Human Caspase 7	25 units
RCH-008	Recombinant Human Caspase 8	25 units
RCH-009	Recombinant Human Caspase 9	25 units
RCH-010	Recombinant Human Caspase 10	25 units
RCH-110S	Recombinant Human Caspase 1-3, 6-10 Set	8 x 25 units

Caspase Substrates

Ready-to-use AFC substrates for caspases

The caspase substrates are AFC-substrates that exhibit both fluorescence and absorbance spectral shifts between the substrate-conjugate and the free dye (AFC). Suitable for fluorescence readers, spectrophotometer, or microtiter plate readers. These substrates are supplied as 1mM ready-to-use solutions and recommended for use at 50µM in assay.

Cat. #	Description	Size
CPS-145	Caspase-1,4,5 Substrate; Ac-WEHD-AFC (1mM)	250µl
CPS-002	Caspase-2 Substrate; Ac-VDVAD-AFC (1mM)	250µl
CPS-370	Caspase-3,7,10 Substrate; Ac-DEVD-AFC (1mM)	250µl
CPS-006	Caspase-6 Substrate; Ac-VEID-AFC (1mM)	250µl
CPS-008	Caspase-8 Substrate; Ac-IETD-AFC (1mM)	250µl
CPS-009	Caspase-9 Substrate; Ac-LEHD-AFC (1mM)	250µl
CPS-013	Caspase-13 Substrate; Ac-LEED-AFC (1mM)	250µl
CPS-113S	Caspase 1-10 and 13 Substrates Set	7 x 250µl

Caspase Inhibitors

Ready-to-use FMK inhibitors for caspases

The inhibitors are potent fluoromethyl ketone (FMK) based, non-toxic, and membrane permeable. Inhibitors are supplied as 1mM ready-to-use solutions and are recommended for use at 1µl/ml cell culture or in caspase assays.

CITED REFERENCES

Leuenroth, S., et al (2008) *Cancer Res.* 68 pp 5257-66

Cat. #	Description	Size
CPI-145	Caspase-1, 4,5 Inhibitor; Z-WEHD-FMK (1mM)	100µl
CPI-002	Caspase-2 Inhibitor; Z-VDVAD-FMK (1mM)	100µl
CPI-370	Caspase-3, 7,10 Inhibitor; Z-DEVD-FMK (1mM)	100µl
CPI-006	Caspase-6 Inhibitor; Z-VEID-FMK (1mM)	100µl
CPI-008	Caspase-8 Inhibitor; Z-LETD-FMK (1mM)	100µl
CPI-009	Caspase-9 Inhibitor; Z-LEHD-FMK (1mM)	100µl
CPI-013	Caspase-13 Inhibitor; Z-LEED-FMK (1mM)	100µl
CPI-00G	Caspase General Inhibitor; Z-VAD-FMK (1mM)	100µl
CPI-113S	Caspase-Family (1-13 & General) Inhibitor Set	8 x 100µl

Cytotoxicity & Cell Proliferation Assays

CytoScan™ LDH Cytotoxicity Assay

The assay quantitatively measures the stable, cytosolic, lactate dehydrogenase (LDH) enzyme, which is released from damaged cells. The released LDH is measured with a coupled enzymatic reaction that results in the conversion of a tetrazolium salt (iodonitrotetrazolium; (INT)) into a red color formazan by diaphorase. The LDH activity is determined as NADH oxidation or INT reduction over a defined time period.

The resulting formazan is measured at 490nm.

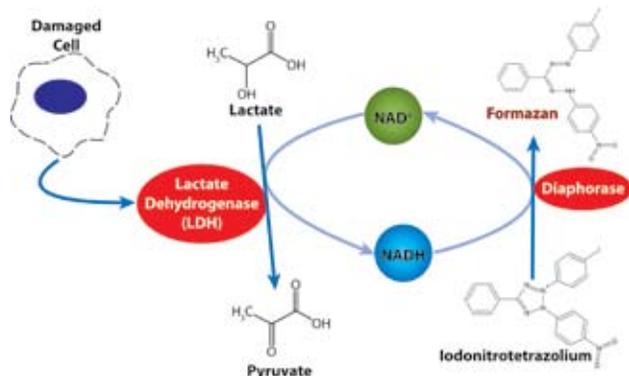


Figure 18: CytoScan™ LDH Cytotoxicity Assay scheme.

FEATURES

- Colorimetric assay
- Quantitatively measures LDH release
- For cell free supernatants from cells in culture (adherent or suspension)

APPLICATIONS

- For the detection of cell toxicity, death, viability or proliferation
- Ideal for high throughput screening

REFERENCES

Haslam, G. et al (2005) *Anal. Biochem.* 336: 187
 Tarnawski, A. (2005) *Biochem. Biophys. Res. Comm.* 333: 207
 Round, J. L et al (2005) *J. Exp. Med.* 201: 419
 Bose, C. et al (2005) *Am. J. Physiol. Gastr. L.* 289: G926
 Chen, A. and Xu, J. (2005) *Am. J. Physiol. Gastr. L.* 288: G447

Cat. #	Description	Size
786-210	CytoScan™ LDH Cytotoxicity Assay	1000 assays

CytoScan™-fluoro Cytotoxicity Assay

A sensitive fluorometric assay for cytotoxicity, proliferation and cell death

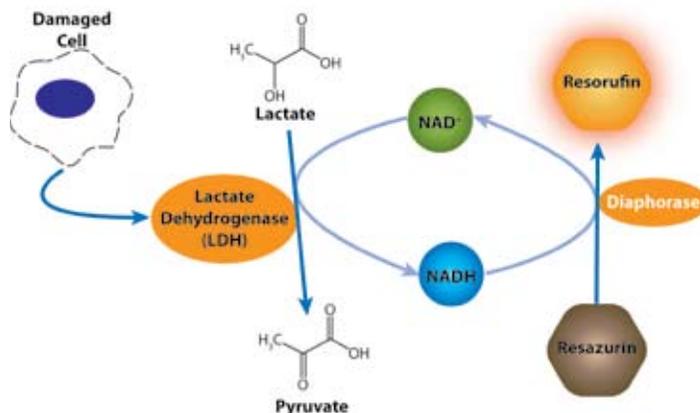


Figure 19: CytoScan™-fluoro cytotoxicity assay scheme

The CytoScan™-fluoro cytotoxicity assay is based on the quantification of cellular lactate dehydrogenase (LDH) released when cells are damaged or under stress. Lactate dehydrogenase released into the culture medium is measured with a diaphorase coupled enzymatic assay that results in the conversion of a non-fluorescent compound (resazurin) to a fluorescent compound (resorufin), which is measured using a fluorometer. The assay detects even low level damage to cell membrane not detected with other methods.

The CytoScan™-fluoro kit does not damage healthy cells and the assay can be performed directly in the cell culture wells containing a mixed population of viable and damaged cells. The kit is supplied with substrate mix, assay buffer, and stop solution.

FEATURES

- Fluorometric assay
- Quantitatively measures LDH release
- Assays performed directly in cell culture wells

APPLICATIONS

- For the detection of cell toxicity, death, viability or proliferation. • Ideal for high throughput screening

REFERENCES

Litterst, C. et al (2009) *Bio-Rad.*
 Haslam, G. et al (2005) *Anal. Biochem.* 336: 187
 Tarnawski, A. (2005) *Biochem. Biophys. Res. Comm.* 334: 207
 Round, J. L et al (2005) *J. Exp. Med.* 201: 419
 Bose, C. et al (2005) *Am. J. Physiol. Gastr. L.* 289: G926
 Chen, A. and Xu, J. (2005) *Am. J. Physiol. Gastr. L.* 288: G447

Cat. #	Description	Size
786-211	CytoScan™-fluoro Cytotoxicity Assay	500 Assays

CytoScan™ WST-1 Cell Proliferation Assay

CytoScan™ WST-1 Cell Proliferation Assay is a sensitive and accurate assay for cell proliferation and cytotoxicity. The assay is highly convenient as it is performed in a single cell tissue culture well and requires no washing, harvesting or solubilization of cells. Adherent or suspension cells are cultured in a microplate and then incubated with WST-1 and the assay is monitored with a spectrophotometer. The assay principle is based upon the reduction of the tetrazolium salt WST-1 to formazan by cellular dehydrogenases. The generation of the dark yellow colored formazan is measured at 420-480nm (optimal at 440nm) and is directly correlated to cell number. The kit components are sufficient for performing up to 500 assays.

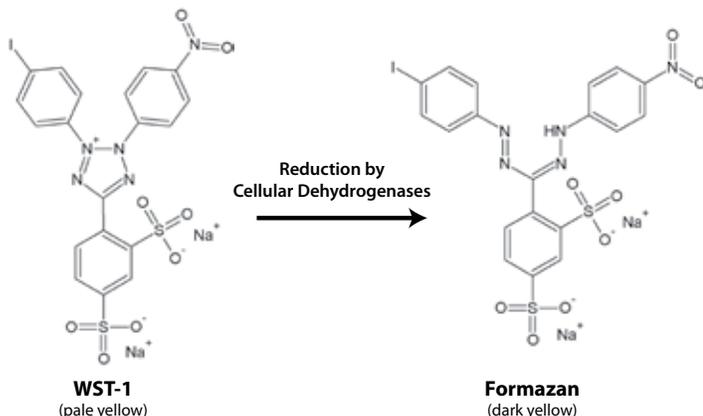


Figure 20: CytoScan™ WST-1 Cell Proliferation assay scheme.

FEATURES

- Colorimetric assay
- Uses WST-1, a high sensitivity tetrazolium salt
- Adherent or suspension cells
- No washing, harvesting or solubilization required

APPLICATIONS

- For the detection of cell toxicity, death, viability or proliferation. Ideal for high throughput screening

Cat. #	Description	Size
786-212	CytoScan™ WST-1 Cell Proliferation Assay	500 assays

CytoScan™ SRB Cytotoxicity assay

To determine total cellular biomass

CytoScan™-SRB Cytotoxicity Assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by sulforhodamine B (SRB). The assay is used for cell density determination, based on the measurement of cellular protein content.

This assay has been used for high-throughput drug screening at the National Cancer Institute (NCI)¹. Sulforhodamine B is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides a sensitive linear response. The color development is rapid and stable and is readily measured at absorbances between 560 and 580nm. The kit components are sufficient for performing up to 1000 assays.

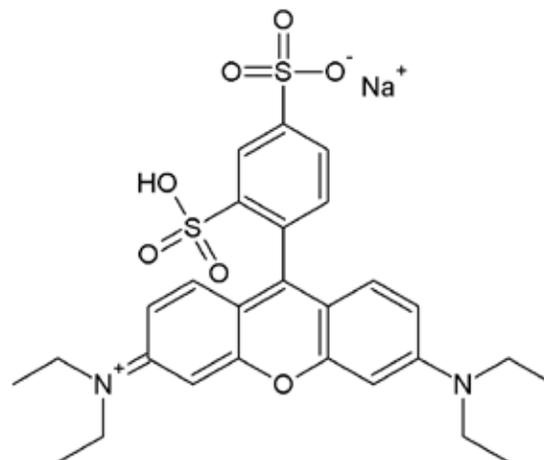


Figure 21: Sulforhodamine B structure.

FEATURES

- Measures total biomass by staining cellular proteins
- Linear response
- Simple, accurate and reproducible assay

APPLICATIONS

- For the detection of cell toxicity, death, viability or proliferation
- Ideal for high throughput screening

REFERENCES

1. Perez, R.P. et al (1993) *Eur. J. Cancer*. 29: 395

Cat. #	Description	Size
786-213	CytoScan™ SRB Cytotoxicity Assay	1000 assays

SAM Methyltransferase Assays

Methylation of key biological molecules and proteins plays important roles in numerous biological systems, including signal transduction, biosynthesis, protein repair, gene silencing and chromatin regulation (1).

The S-adenosylmethionine (SAM) dependent methyltransferases use SAM, the second most commonly used enzymatic cofactor after ATP. SAM, also known as AdoMet, acts as a donor of a methyl group that is required for the modification of proteins and DNA. Aberrant levels of SAM have been linked to many abnormalities, including Alzheimer's, depression, Parkinson's, multiple sclerosis, liver failure and cancer (2).

The G-Biosciences' SAM Methyltransferase Assays are continuous enzyme coupled assays that can continuously monitor SAM-dependent methyltransferases (3) without the use of radioactive labels or endpoint measurements. A sensitive, UV-based, SAM265, and a colorimetric, SAM510, SAM Methyltransferase assays are offered.

REFERENCES

- Dorgan, K.M. et al. (2006) *Anal. Biochem.* 350:249-255
 Schubert, H.L. et al. (2003) *Trends Biochem. Sci* 28: 329-335
 Cheng, X. & Blumenthal, R.M. (1999) *S-Adenosylmethionine Dependent Methyltransferases*
 World Scientific, Singapore

SAM265™

A sensitive, UV range, continuous enzyme coupled assay

Basically, the removal of the methyl group from SAM, by the SAM methyltransferase, generates S-adenosylhomocysteine, which is rapidly converted to S-ribosylhomocysteine and adenine by the included adenosylhomocysteine nucleosidase. This rapid conversion prevents the buildup of adenosylhomocysteine and its feedback inhibition on the methylation reaction. Finally, the adenine is converted to hypoxanthine by adenine deaminase, the second enzyme in the assay. The rate of production of hypoxanthine is measured by absorbance change.

The assay can be adapted to be used with any SAM dependent methyltransferase or an enzyme reaction that produces S-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of adenosylhomocysteine nucleosidase.

The kit is supplied with an enzymatic and chemical positive control to determine the ideal assay conditions and with enough reagents for 100 microwell assays.

FEATURES

- Detection of protein methylation or screening methylation inhibitors
- Continuous enzyme coupled assay
- Supplied with all reagents, including positive control
- Adaptable for enzymes that generate S-adenosylhomocysteine or 5'-methylthioadenosine

APPLICATIONS

- For the kinetic analysis of protein SAM methyltransferase enzymes
- Ideal for screening of methyltransferase inhibitors

REFERENCES

- Aktas, M. and Narberhaus, F. (Apr 2009) *J. Bacteriol.* 191: 2033-2041
 Dorgan, K.M. et al. (2006) *Anal. Biochem.* 350:249-255
 Schubert, H.L. et al. (2003) *Trends Biochem. Sci* 28: 329-335
 Cheng, X. & Blumenthal, R.M. (1999) *S-Adenosylmethionine Dependent Methyltransferases*
 World Scientific, Singapore

Cat. #	Description	Size
786-425	SAM265™ SAM Methyltransferase Assay	100 Assays

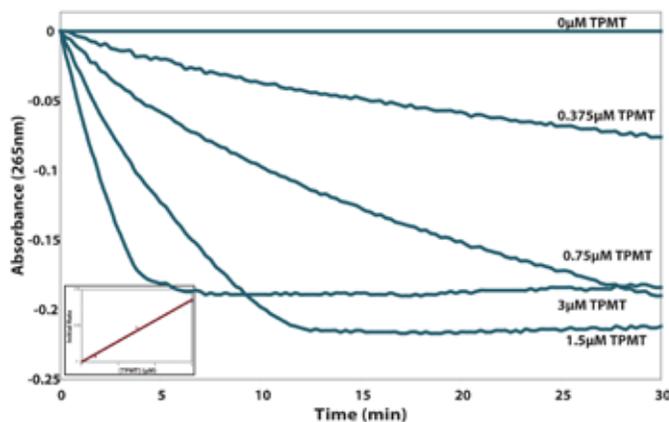


Figure 22: SAM265™ methyltransferase assay quantitatively assays Thiopurine S-methyltransferase (EC 2.1.1.67) (TPMT). 0-3µM TPMT was assayed with SAM265, using thiophenol as a substrate. The inset graph shows a linear correlation between absorbance change at 265nm and TPMT concentration.

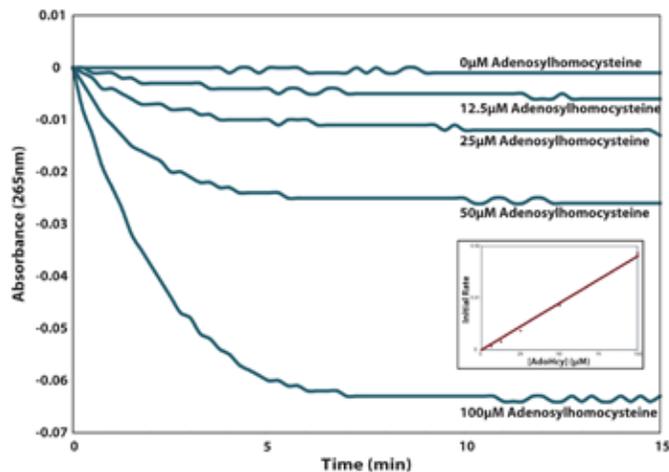


Figure 23: SAM265™ methyltransferase assay quantitatively measures Adenosylhomocysteine (AdoHcy). 0-100µM AdoHcy was assayed with SAM265. The inset graph shows that there is a linear correlation between absorbance change at 265nm and AdoHcy concentration.

SAM510™

A colorimetric, continuous enzyme coupled assay

The SAM510™: SAM Methyltransferase Assay is a continuous enzyme coupled assay that can continuously monitor SAM-dependent methyltransferases (1) without the use of radioactive labels or endpoint measurements.

Basically, the removal of the methyl group from SAM generates S-adenosylhomocysteine, which is rapidly converted to S-ribosylhomocysteine and adenine by the included adenosylhomocysteine nucleosidase. This rapid conversion prevents the buildup of adenosylhomocysteine and its feedback inhibition on the methylation reaction. Finally, the adenine is converted to hypoxanthine, by adenine deaminase, which in turn is converted to urate and hydrogen peroxide. The rate of production of hydrogen peroxide is measured with a colorimetric assay by an increase in absorbance at 510nm.

The assay can be adapted to be used with any SAM dependent methyltransferase or an enzyme reaction that produces S-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of adenosylhomocysteine nucleosidase.

The kit is supplied with a chemical positive control to determine the ideal assay conditions and with enough reagents for 100 microwell assays.

FEATURES

- Detection of protein methylation or screening methylation inhibitors
- Continuous enzyme coupled assay for kinetic studies
- Colorimetric, non radioactive assay
- Supplied with all reagents, including positive control
- Adaptable for enzymes that generate S-adenosylhomocysteine or 5'-methylthioadenosine

APPLICATIONS

- For the kinetic analysis of protein SAM methyltransferase enzymes
- Ideal for screening of methyltransferase inhibitors

REFERENCES

Dorgan, K.M. et al. (2006) *Anal. Biochem.* 350:249-255

Cat. #	Description	Size
786-430	SAM510™: SAM Methyltransferase Assay	100 Assays

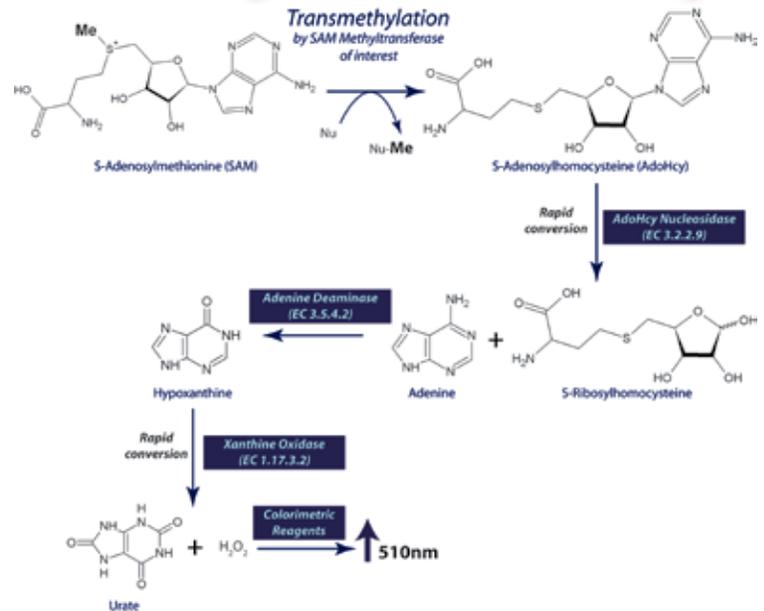


Figure 24: The SAM510™ Methyltransferase Assay scheme.

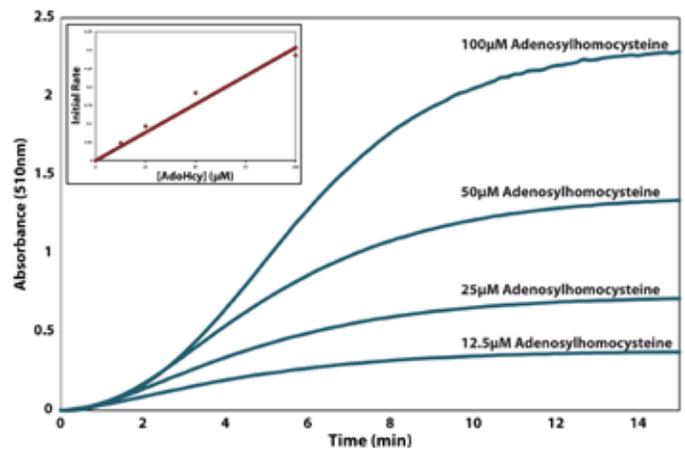


Figure 25: SAM510™ Assay quantitatively assays Adenosylhomocysteine (AdoHcy). 0-100µM AdoHcy was assayed with SAM510™. The inset graph shows that there is a linear correlation between absorbance change at 510nm and AdoHcy concentration.

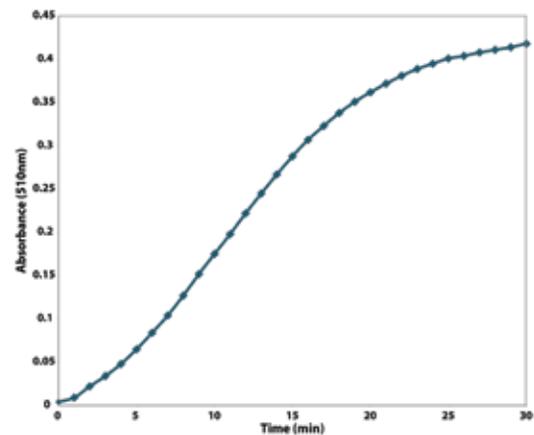


Figure 26: Human lysine specific histone Methyltransferase SET7/9 assayed with 20µM TAF-10 as the acceptor substrate by SAM510™.

SAM Methyltransferase Assays

SAMfluoro™

A fluorescent, continuous enzyme coupled assay

The SAMfluoro: SAM Methyltransferase Assay is a fluorescent, continuous enzyme coupled assay that can continuously monitor SAM-dependent methyltransferases (1) without the use of radioactive labels or endpoint measurements.

The removal of the methyl group from SAM generates S-adenosylhomocysteine (AdoHcy), which is rapidly converted to S-ribosylhomocysteine and adenine by the included AdoHcy nucleosidase. This rapid conversion prevents the buildup of AdoHcy and its feedback inhibition on the methylation reaction. Finally, the adenine is converted to hypoxanthine, by adenine deaminase, which in turn is converted to urate and hydrogen peroxide (H_2O_2). The rate of production of hydrogen peroxide is measured with 10-acetyl-3,7,-dihydroxyphenoxazine (ADHP), which produces the highly fluorescent compound resorufin. Resorufin production can easily be measured with an excitation wavelength of 530-540nm and an emission wavelength of 585-595nm.

Figure 21 shows the resorufin standard curve generated from the supplied standards and figure 22 shows the assay of human lysine specific histone methyltransferase Set7/9 assayed with 20 μ M TAF-10 as the acceptor substrate.

The kit is supplied with enough reagents for 100 microwell assays. The assay is supplied with AdoHcy as a positive control. The assay can be adapted to be used with any purified SAM dependent methyltransferase or a purified enzyme that produces S-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of AdoHcy nucleosidase.

FEATURES

- Detection of protein methylation or screening methylation inhibitors
- Continuous enzyme coupled assay for kinetic studies
- Fluorescent, non radioactive assay
- Supplied with all reagents, including positive control
- Adaptable for enzymes that generate S-adenosylhomocysteine or 5'-methylthioadenosine

APPLICATIONS

- For the kinetic analysis of protein SAM methyltransferase enzymes
- Ideal for screening of methyltransferase inhibitors

REFERENCES

Dorgan, K.M. et al. (2006) *Anal. Biochem.* 350:249-255

Cat. #	Description	Size
786-431	SAMfluoro™: SAM Methyltransferase Assay	100 Assays

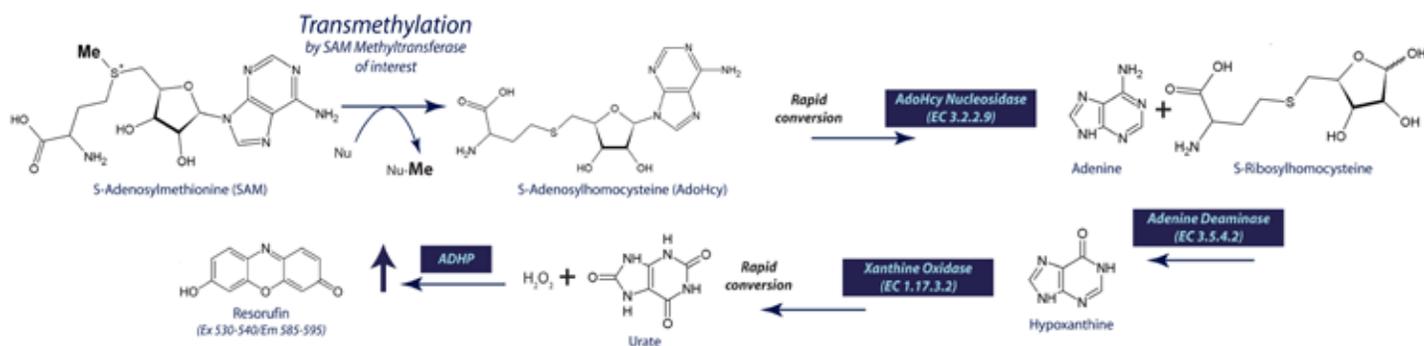


Figure 29: The scheme for SAMfluoro™ assay.

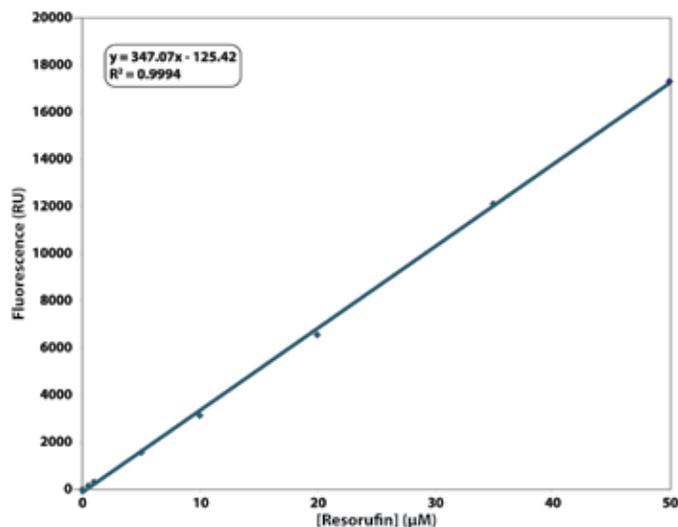


Figure 27: SAMfluoro™: SAM Methyltransferase Assay Resorufin Standard Curve.

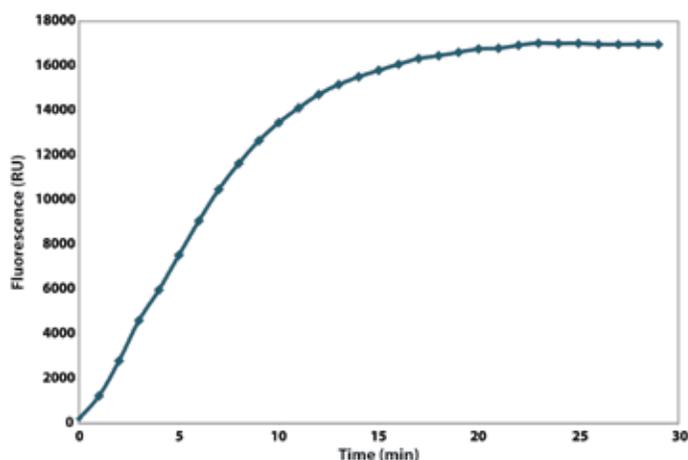


Figure 28: Human lysine specific histone methyltransferase Set7/9 assayed with 20 μ M TAF-10 as the acceptor substrate.

G-Biosciences Product Line Overview

Protein Research

Estimation

7 Assays

Isolation

Extraction & Lysis

Fractionation & Enrichment

Sample Preparation

Reagents

Electrophoresis

Detection

Western Blotting

Mass Spectrometry

Assays (ELISA)

Purification

Affinity Resins

Activated Resins

Antibody Purification

Modification

Labeling

Crosslinkers
Reducing Agents
Alkylating Agents
Protein Cleavage
Iodination
Amino Acid Side Chain Modifiers

Antibody

Production

Purification

Fragmentation

SAM Methyltransferase

Cell Toxicity & Proliferation

Apoptosis

Protease

Phosphatase
Peroxide

B-Galactosidase

Continuous, Enzymatic Assays
Lactate Dehydrogenase (LDH)
SRB
WST-1

Caspase

Inducers
Assays
Inhibitors

CPRG
Fluorescent (MUG)

Genomic DNA

Plasmid DNA

Electrophoresis

PCR

RNA

Yeast

Isolation

Isolation
Colony Screening
Transformation
Apparatus
Loading Dyes
DNA Ladders
Gel Extraction
Tag
dNTPs
Extraction
RNase Decontamination
Transformation
Plasmid Isolation

CB-X
Non Interfering
SPN
RED 660
dotMETRIC
BCA
CB
Sample Grinding

Lysis Buffers

12 Fractionation Kits
Dialysis (Micro)
Concentration
Contamination Removal

Protease Inhibitors

Detergents
Chaotropes

1D & 2D Reagents

Gel Stains

1 Hour System

Blocking Agents

Secondary Antibodies
Chemiluminescence Detection
Trypsin, Mass Spec Grade
InGel Kits
Coated Plates

Blocking Agents

Secondary Antibodies
Detection Reagents

6X His Tag

GST Tag
Biotin Tag
CBP Tag
Sulphydryl reactive
Amine reactive
Carboxyl reactive
Drug/ Steroid reactive
Protein A or G
Pearl Resin
Biotin
Fluorescent Dye
Enzyme (HRP/AP)

Mild Denaturing
Strong Chaotropic
Specialized

Desalting
Detergent Removal
General Cocktails
Species Specific
Individual Inhibitors

2D Specific Kits
Buffers & Reagents
Coomassie
Silver
Reversible

Non-Animal
Animal
Non-Protein

Non-Animal
Animal
Non-Protein

Nickel resin
Cobalt resin
Copper resin
Zinc Resin
Glutathione Resin
Streptavidin Resin
Calmodulin Resin

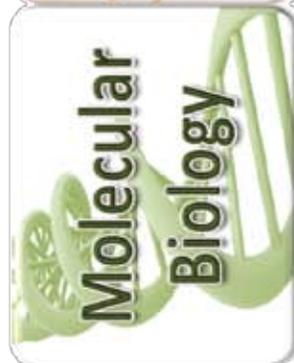
BSA
KLH
HyperCarrier

Carrier Proteins

Peptide Coupling
Protein A or G Resin
Activated Resins
Pearl Resin
Thiophilic Resin
Ficin
Pepsin
Papain

Assays
Substrates
Inhibitors

Tissue
Blood
Plant
Yeast
Bacteria
Fungi
Mouse Tail





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