G-Biosciences

Protein Assays

Handbook &

Selection Guide

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

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

Dye Binding Assays (Bradford)

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 (A_{max}= 465nm) to blue (A_{max}= 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.

CB™ and CB-X™ protein assays are dye binding protein assays.

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

Copper Ion Based Assays (Lowry & BCA)

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 (Figure 1). Protein assays based on copper ions can be divided into two groups, assays that detect reduced cuprous ions (Cu^{+}) and assays that detect the unbound cupric (Cu^{2+}) ions.

The cuprous ions are detected either with bicinchoninic acid (BCA) or Folin Reagent (phosphomolybdic/ phosphotungstic acid) as in the protein assays based on Lowry method. Cuprous ions (Cu^{+}) reduction of Folin Reagent produces a blue color that can be read at 650-750nm. The amount of color produced is proportional to the amount of peptide bonds, i.e. size as well as the amount of protein/peptide.

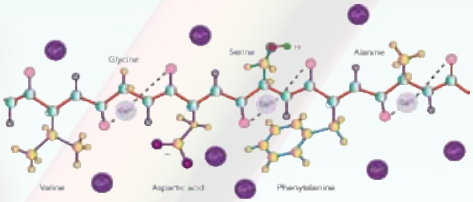


Figure 1: The interaction of copper ions with proteins.

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.

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 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 (Figure 2). By measuring the diameter of the protein spots with a predeveloped measuring gauge, the amount of protein can be estimated.

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

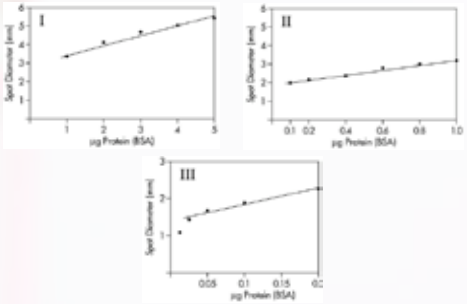


Figure 2: The linear relationship of protein BSA concentration with the protein spot diameters.

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, containing 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 (Figure 3). 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™) Protein Assays, CB-X™, SPN™ and SPN™-htp are designed to first remove non-protein agents from the protein solutions. The dotMETRIC™ protein assay on the other hand is designed to circumvent the interfering effects of non-protein agents in the protein solution.

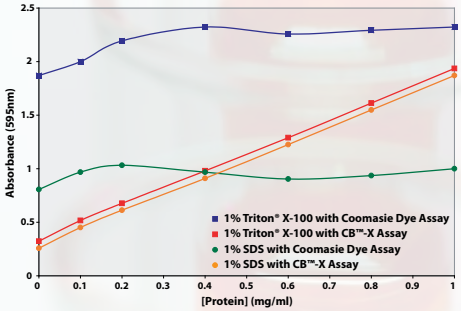


Figure 3: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton X-100 (blue) or 1% SDS (green) were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton X-100 (red) or 1% SDS (orange) were assayed using 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.

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™ 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 signification protein-to-protein variation (see figure 5). 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™ 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™ Protein Assay

A single tube assay for rapid estimation and without preparing any calibration plot.

CB™ Protein Assay

A Coomassie dye based protein assay.

NI™ (Non-Interfering™) Protein Assay

An assay that overcomes interference of agents commonly present in protein solutions.

SPN™ Protein Assay

A spin format protein assay for single sample or high throughput screening.

Protein dotMETRIC™

A 1µl Protein Assay. A test strip based protein assay that takes less than 10 minutes.

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 that interfere with accurate protein estimation. These reagents include detergents, chaotropes, reducing agents, alkylating agents, sugars, high salt concentrations, buffering agents and chelating agents (Table 1). 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.

Figure 4 depicts the simple scheme of CB-X™ Protein Assay. 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 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.

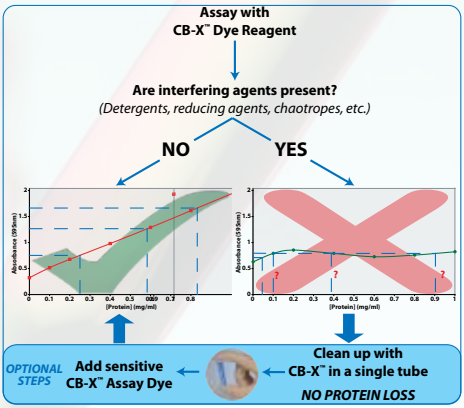


Figure 4: CB-X™ Protein Assay Scheme. Protein samples are rapidly assayed with a sensitive, single reagent with in five minutes. In most cases the assay will have a linear response (Left side), however if interfering agents are present an artifactual curve or results will be produced (Right side). In this situation the samples are cleaned up with the supplied reagents and assayed in a single tube. This generates accurate protein estimations.

A set of bovine serum albumin standards are supplied for generating curves when using CB-X™ Assay Dye alone or for researcher's 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.

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 (Ref. 1-6) 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.

CITED APPLICATIONS

1. *Kump, D.S. and Booth, F.W. (2005) J. Physiol. 565: 911 915.*
2. *Deszo, E.L. et al. (2004) PNAS. 102: 5564-5569.*
3. *Cui, Y. et al. (2005) J. Bacteriol. 187: 4792-4803.*
4. *Chatterjee, A. et al. (2005) J. Bacteriol. 187: 8026-8038.*
5. *Manikandan, K. et al. (2005) Acta Cryst. 61: 747-749.*
6. *Saradhi, M. et al. (2005) Cell Res. 15: 785-795.*

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 (see Table 2).

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.

Tolerance to 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

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

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

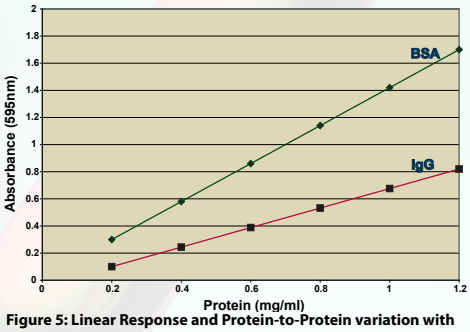


Figure 5: Linear Response and Protein-to-Protein variation with CB™ Protein Assay.

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.

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 (see tables 3 and 4).

The NI™ Protein Assay is composed of two simple steps (see figure 6):

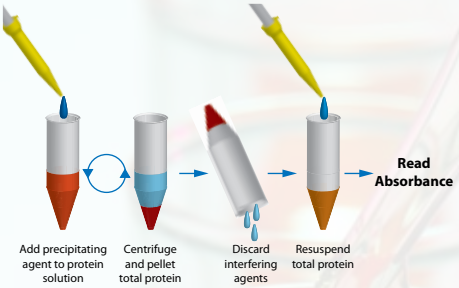


Figure 6: The *NI*™ Protein Assay Scheme

Non-Interfering™ Protein Assay Overview:

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 (see figure 7). The color density is inversely proportional to the amount of protein.

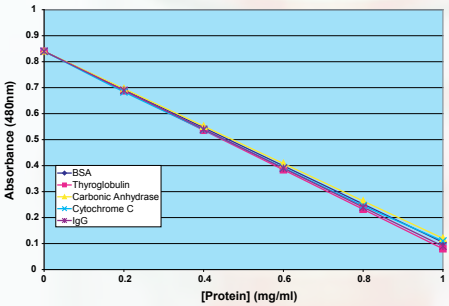


Figure 7: NI™ Protein Assay shows minimal protein-to-protein variation. BSA, thyroglobulin, carbonic anhydrase, cytochrome C and bovine immunoglobulin G were assayed for protein-to-protein variation. The proteins produced identical color and slope in the range of 0.5µg-50µg, giving an average ratio of 1.01.

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.

CITED APPLICATIONS

1. *Ho, T.H., et al (2005) Hum. Mol. Genet. 14:1539-47.*
2. *Loeb, D.M. et al (2002) J. Biol. Chem. 277: 19627-32.*
3. *Gushwa, N.N., et al (2003) Plant Physiol. 132: 1925-40.*
4. *Reeve, R., et al (2002) PNAS. 99: 8608-8616.*

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

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 4: NI™ Protein Assay is compatible with strong chaotropic extraction buffers

Cat. #	Description	Size
786-005	NI™ (Non-Interfering™) Protein Assay Kit with Albumin Standard	500 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)

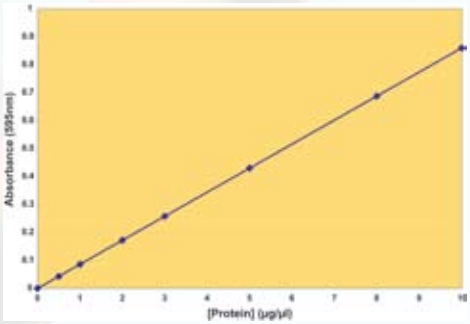


Figure 8: Standard Calibration Plot for SPN™ Protein Assay.

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.

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.

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

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

Protein dotMETRIC™ Assay

1µl Assay For Rapid Protein Estimation

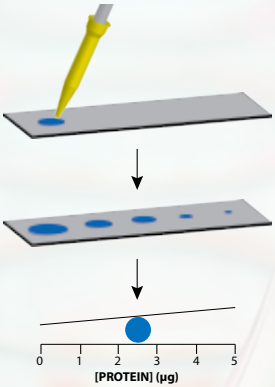


Figure 9: Protein dot METRIC™ Protein Assay Scheme.

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 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 (see figure 9). No expensive spectrophotometers or cuvettes required.

Easy To Use Spot Application Device

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.

No Protein-To-Protein Variation

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.

Resistant To Most Common Laboratory Agents

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.

DLD™ PROTOCOL

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™) (figure 10).

This dilution factor is used for the determination of protein concentration.

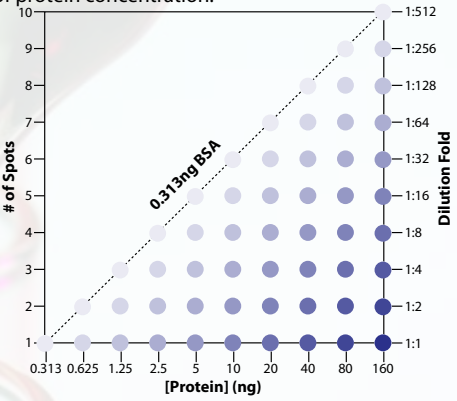


Figure 10: A representation of the DLD™ Protocol.

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.

CITED APPLICATIONS

1. *Pandey, D.G., et al (2006) Blood. 107: 575*
2. *Tomasek, J.J., el alb(2006) Invest. Opthal. Vis. Sci. 47: 2693*
3. *Vallon, M. and Essler, M. (2006) J. Biol. Chem. 281: 34179*
4. *Fujimoto, Y. et al. (2007) J. Lipid Res. 48:1280*

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 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. They make for faster and more reliable protein quantitation. The Prediluted Protein Standards are supplied in 6 x 5ml aliquots ranging from 0.1mg/ml-1.0mg/ml.

Bovine γ-Globulin Protein Standards

γ-Globulin standard (2mg/ml) prepared in saline buffer. The standard is supplied as 2x5ml aliquot.

Prediluted Bovine γ-Globulin Protein Standards

γ-Globulin protein standards in an easy-to-use prediluted format. They make for faster and more reliable protein quantitation. The Prediluted Protein Standards are supplied in 6x5ml 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.

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
786-114G	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
786-63	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

Selection Guide for Protein Assays

	Interfering Agent Compatible	Interfering Agents Removed	Protein-To-Protein Variation	Tubes Required For Assay	Sample Volume	Linear Response Between	Assay Time (~mins)	Calibration Plot Required	Instrument Required
CB-X™ (786-12X)	Yes	Yes	Yes	One per sample	5-100µl	0.5-50µg	10	No	Spectrophotometer, centrifuge
CB™ (786-012)	Limited	No	Yes	For Samples & Standards	5-100µl	0.5-50µg	30	Yes	Spectrophotometer
NI™ (786-005)	Yes	Yes	Minimal	For Samples & Standards	1-50µl	0.5-50µg	30	Yes	Spectrophotometer, centrifuge
SPN™ (786-020)	Yes	Yes	Yes	One per sample	1-10µl	0.5-10µg	10	No	Spectrophotometer, centrifuge
SPN™-*htp* (786-021)	Yes	Yes	Yes	One per sample	1-10µl	0.5-10µg	10	No	Spectrophotometer, centrifuge
***dot*METRIC™** (786-20)	Yes	No	Minimal	No	1µl	25ng-1µg	10	No	None

Other Protein Assays

G-Biosciences offers a range of assays to measure specific proteins, including phosphatases, proteases, capases, apoptosis-related and SAM methyltransferases.

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 (Figure 11).

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.

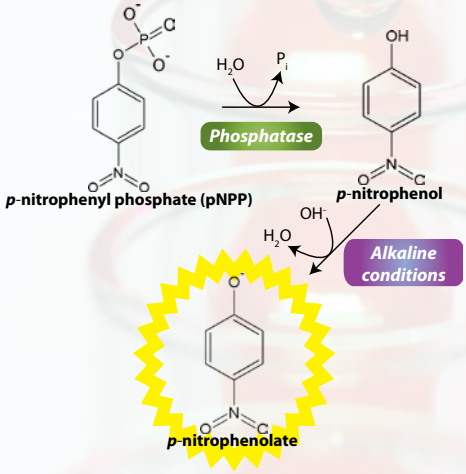


Figure 11: The Phosphatase Assay Scheme

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

ProteSEEKER™

Identify Destructive Proteases

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

*Prote*SEEKER™ 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, *Prote*SEEKER™ can be used to test existing protease inhibitor cocktails and identify their inadequacies and therefore supplement in additional protease inhibitors.

*Prote*SEEKER™ 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.

CITED REFERENCES

1. Person, M.D. et al (2006) J. Biomol. Tech. 17: 145.
2. 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

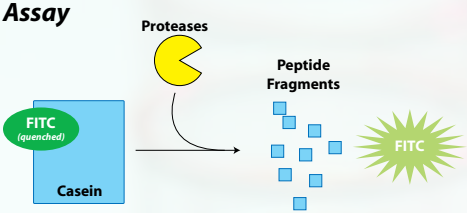


Figure 12: *Fluoro*™ Protease Assay Scheme

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.

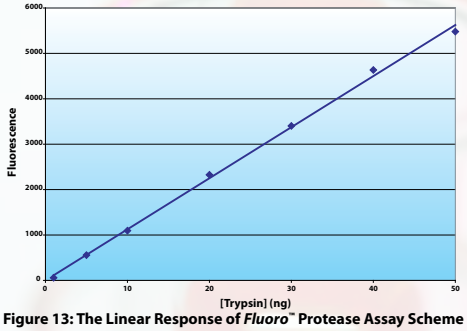


Figure 13: The Linear Response of *Fluoro*™ Protease Assay Scheme

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

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-trifluromethyl 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

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.

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.

CITED REFERENCES

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

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 (see figure below). 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.

FEATURES

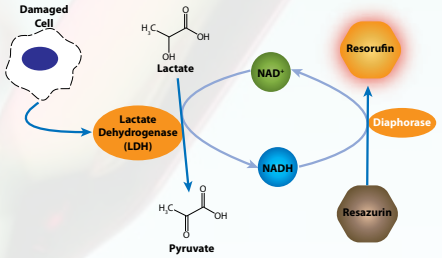
- Colorimetric assay.
- Uses WST-1, a high sensitivity tetrazolium salt.
- Adherent or suspension cells.
- No washing, harvesting or solubilization required.

APPLICATIONS

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

CytoScan™-fluoro Cytotoxicity Assay

A sensitive fluorometric assay for cytotoxicity, proliferation and cell death



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.

CITED REFERENCES

1. Haslam, G. et al (2005) Anal. Biochem. 336: 187

Cat. #	Description	Size
786-210	CytoScan™ LDH Cytotoxicity Assay	1000 Assays
786-212	CytoScan™ WST-1 Cell Proliferation Assay	500 Assays
786-211	CytoScan™-*fluoro* Cytotoxicity Assay	500 Assays

For other apotosis related assays and reagents visit www.GBiosciences.com

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

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

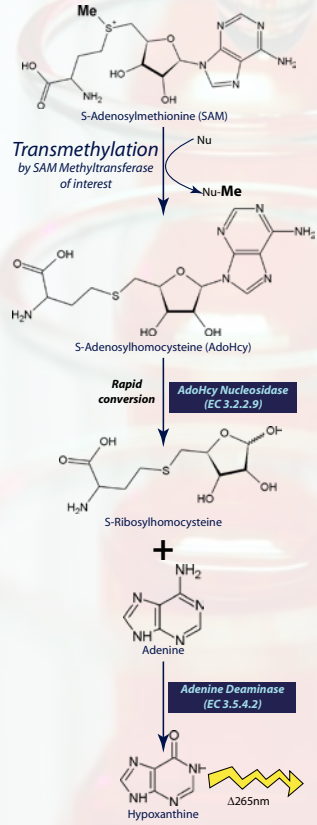


Figure 14: SAM265™: SAM Methyltransferase Assay Scheme.

SAM265™

A sensitive, UV range, continuous enzyme coupled assay

Figure 13 outlines the general scheme of the 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 5-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of adenosylhomocysteine nucleosidase.

The kit is supplied with an enzymatic (figure 15) and chemical (figure 16) positive control to determine the ideal assay conditions and with enough reagents for 100 microwell assays.

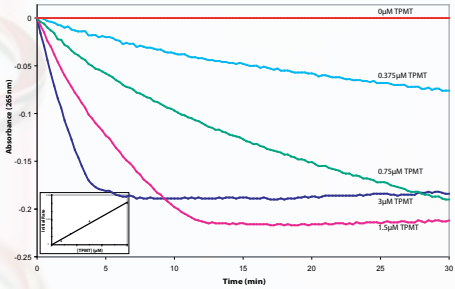


Figure 15: SAM265™ Assay quantitatively assays Thiopurine S-methyltransferase (EC 2.1.1.67) (TPMT). 0-3μM TPMT was assayed with SAM265, using thiophenol as a substrate. The inset graph shows a linear correlation between absorbance change at 265nm and TPMT concentration.

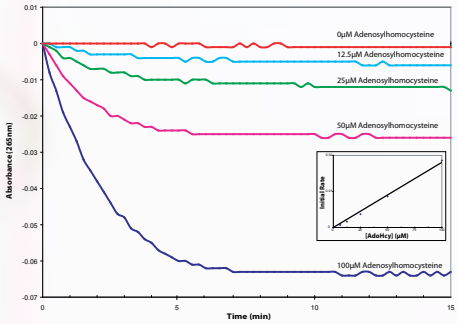


Figure 16: SAM265™ Assay quantitatively assays Adenosylhomocysteine (AdoHcy). 0-100μM AdoHcy was assayed with SAM265. The inset graph shows that there is a linear correlation between absorbance change at 265nm and AdoHcy concentration.

FEATURES

- Detection of protein methylation or screening methylation inhibitors.
- Continuous enzyme coupled assay.
- Supplied with all reagents, including positive control, positive control enzyme and substrate.
- 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.

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

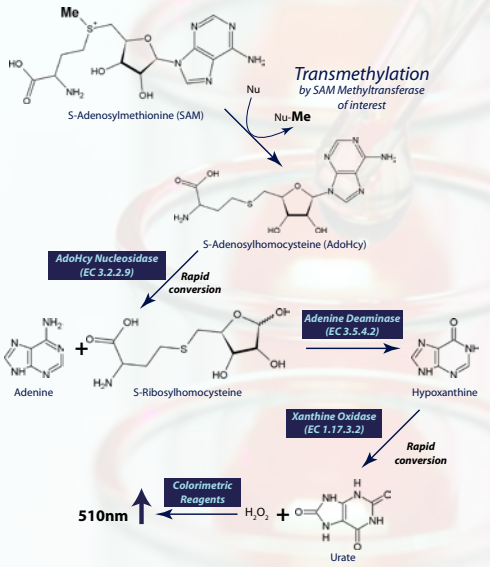


Figure 17: The SAM510™ Methyltransferase Assay Scheme

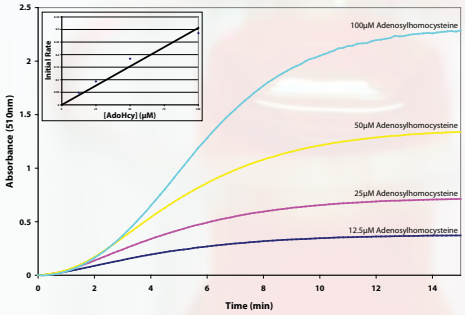


Figure 18: SAM510™ Assay quantitatively assays Adenosylhomocysteine (AdoHcy). 0-100μM AdoHcy was assayed with SAM510™. The inset graph shows that there is a linear correlation between absorbance change at 510nm and AdoHcy concentration.

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.

Figure 17 outlines the general scheme of the assay. 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 5-adenosylhomocysteine or 5′-methylthioadenosine, due to the specificity of adenosylhomocysteine nucleosidase.

The kit is supplied with a chemical (figure 18) 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, positive control enzyme and substrate.
- 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

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

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