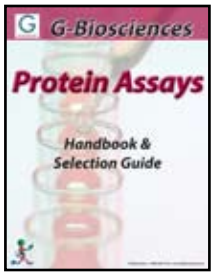
G-Biosciences

Bioassays

Handbook & Selection Guide



- *Protein Estimation Assays*
- *Apoptosis Assays*
- *Cytotoxicity Assays*
- *SAM Methyltransferase Assays*
- *Protease Assays*
- *Phosphatase Assays*
- *Peroxide Assay*

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- *Protein Fractionation Kits*
- *Dialysis (Micro) System*
- *Electrophoresis Clean-Up*
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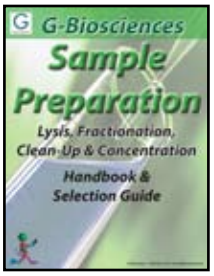

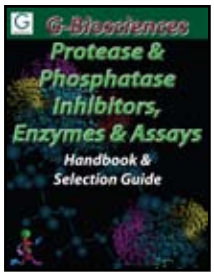

- *Protease Inhibitor Cocktails*
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- *Proteomic Grade Detergents*
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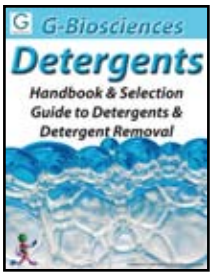
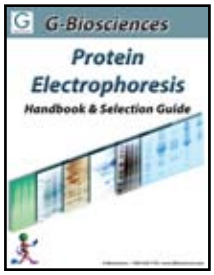
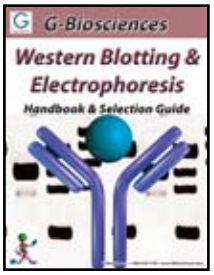
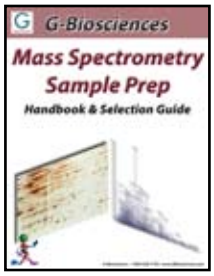




- *Gel Preparation Chemicals*
- *Protein Marker Ladders*
- *Electrophoresis Buffers*
- *Reducing & Alkylating Reagents*
- *Protein Gel Stains*

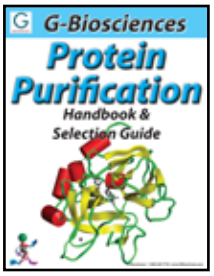
- *1-Hour Western System*
- *Transfer Buffers & Membranes*
- *Membrane Stains*
- *Blocking Buffers*
- *Secondary Antibodies*
- *Detection Reagents*
- *Reprobing Reagents*

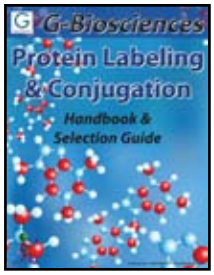




- *Protein Sample Preparation*
- *Protein Clean-Up Systems*
- *Electrophoresis Reagents*
- *Mass Spec Grade Protease*
- *InGel Digestion Kits*
- *Peptide Generation Reagents*

- *Affinity Resins*
- *6X His Protein Purification Kits*
- *GST Protein Purification Kits*
- *Antibody Purification*
- *Activated Resins*
- *Buffers & Reagents*





- *Biotin Labeling*
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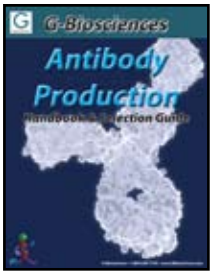
- *Agarose Coupling Kits*
- *Fluorescent Dye Labeling Kits*
- *Enzyme Labeling Systems*

- *Carrier Proteins*
- *Peptide Coupling Systems*
- *Antibody Purification Resins*
- *Antibody Fragmentation Kits*



- *Coated Plates*
- *Blocking Buffers*
- *Wash Buffers*
- *Secondary Antibodies*
- *Detection Reagents*
- *Antibody Labeling Systems*

- *Homobifunctional*
- *Heterobifunctional*
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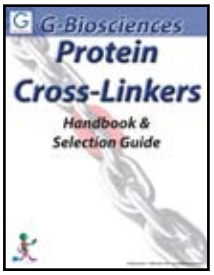
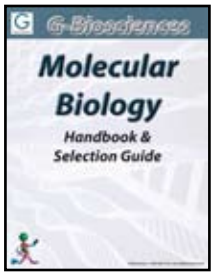
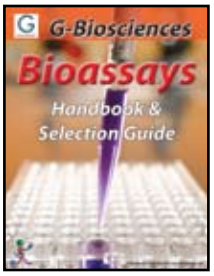
- *Cross-Linking Systems*





- *DNA Isolation*
- *Transformation & Screening*
- *Polymerase Chain Reaction*
- *Agarose Electrophoresis*
- *RNA Isolation*
- *Yeast Transformation*

- *Apoptosis Assays*
- *Cytotoxicity Assays*
- *SAM Methyltransferase Assays*
- *Protease Assays*
- *Phosphatase Assays*
- *Peroxide Assay*
- *ELISA*



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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 5-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

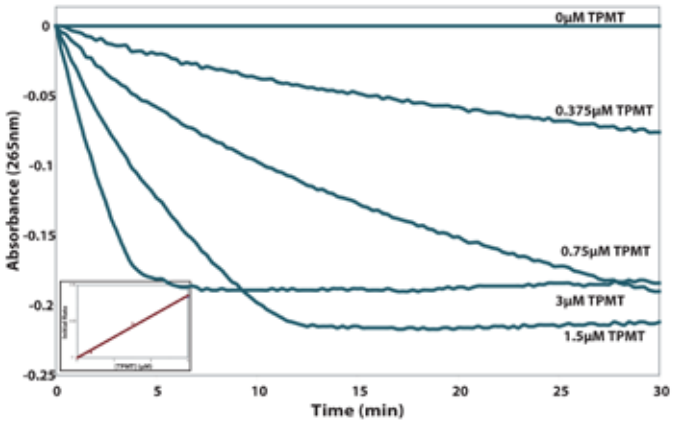

Figure 1: SAM265™ methyltransferase assay quantitatively assays Thiopurine S-methyltransferase (EC 2.1.1.67) (TPMT). 0-3µM TPMT was assayed with SAM265, using thiophenol as a substrate. The inset graph shows a linear correlation between absorbance change at 265nm and TPMT concentration.

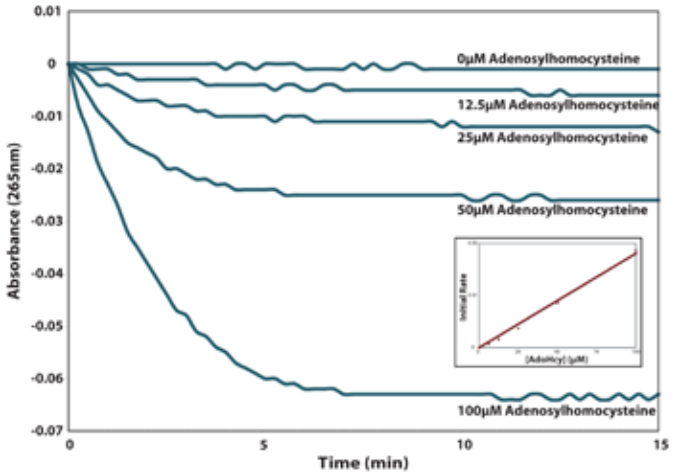

Figure 2: SAM265™ methyltransferase assay quantitatively measures Adenosylhomocysteine (AdoHcy). 0-100µM AdoHcy was assayed with SAM265. The inset graph shows that there is a linear correlation between absorbance change at 265nm and AdoHcy concentration.

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 5-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

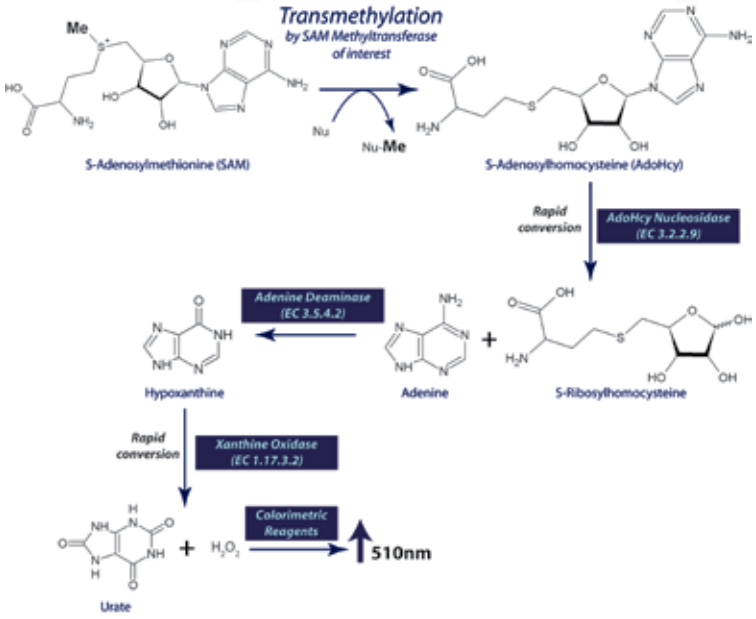

Figure 3: The SAM510™ Methyltransferase Assay scheme.

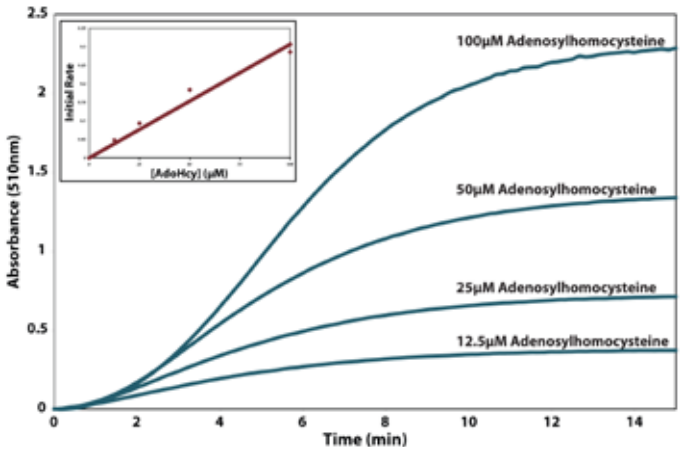

Figure 4: SAM510™ Assay quantitatively assays Adenosylhomocysteine (AdoHcy). 0-100µM AdoHcy was assayed with SAM510™. The inset graph shows that there is a linear correlation between absorbance change at 510nm and AdoHcy concentration.

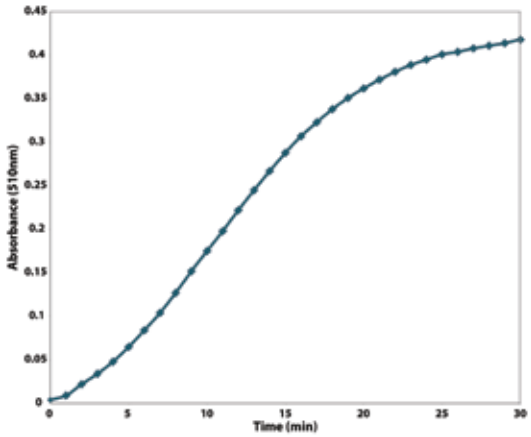

Figure 5: Human lysine specific histone Methyltransferase SET7/9 assayed with 20µM TAF-10 as the acceptor substrate by SAM510™.

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 20µM 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 5-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 Assayy	100 Assays

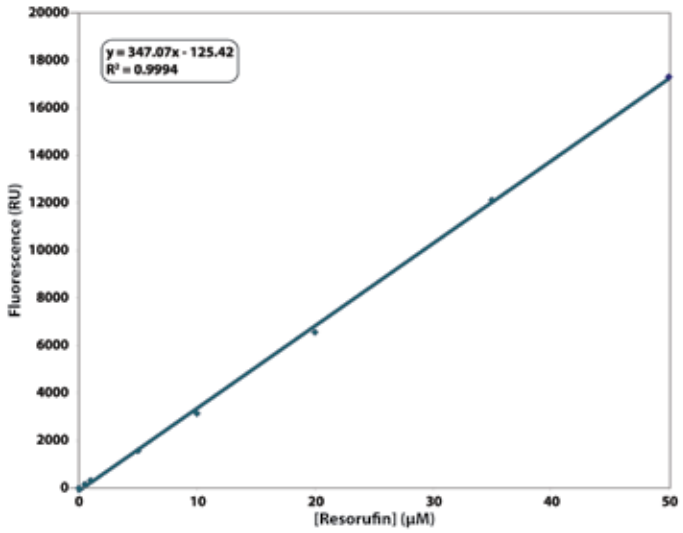

Figure 6: SAMfluoro™: SAM Methyltransferase Assay Resorufin Standard Curve.

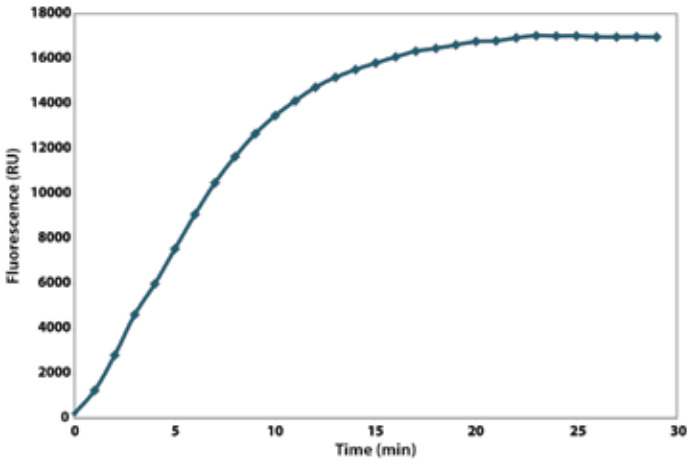

Figure 7: Human lysine specific histone methyltransferase Set7/9 assayed with 20µM TAF-10 as the acceptor substrate.

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.

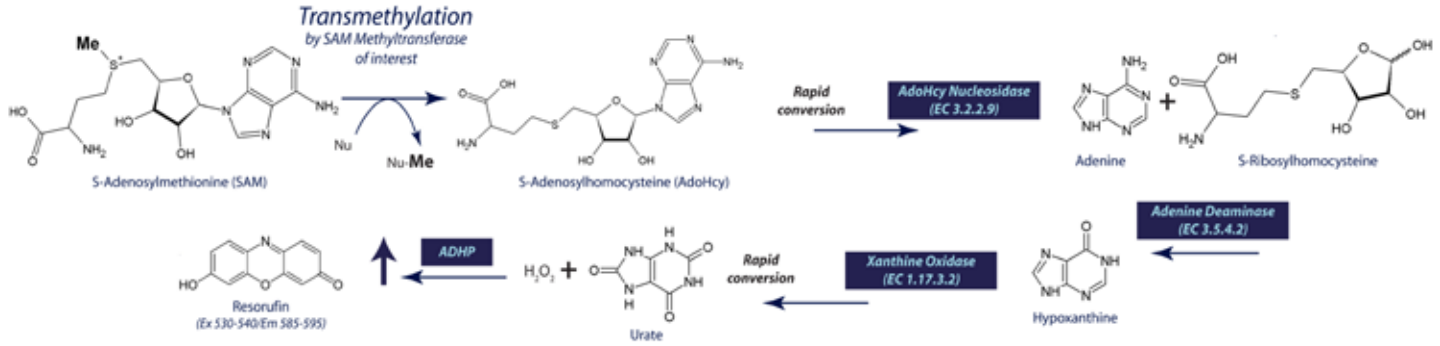

Figure 29: The scheme for SAMfuoro™ assay.

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

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

Dye Binding Assays

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

RED 660™, CB™ 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.

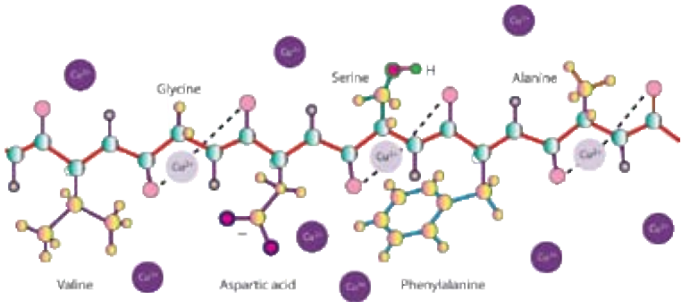

Figure 8: The interaction of copper ions with proteins.

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

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

Non-Interfering™ (NI™) Protein Assay is based on the detection of unbound cupric ions (Cu^{2+}) under alkaline condition. Three BCA (bicinchoninic acid) assays are offered.

Test Strip Based Protein Assay

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

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

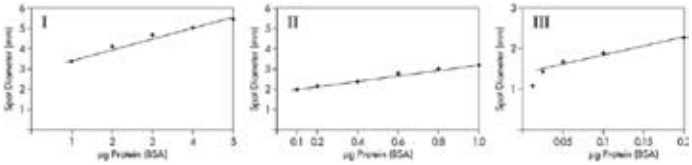

Figure 9: The linear relationship of protein BSA concentration with the protein spot diameters.

PROTEIN ASSAY SELECTION

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

Interfering Agents

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

Non-Interfering™ (NI™) 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.

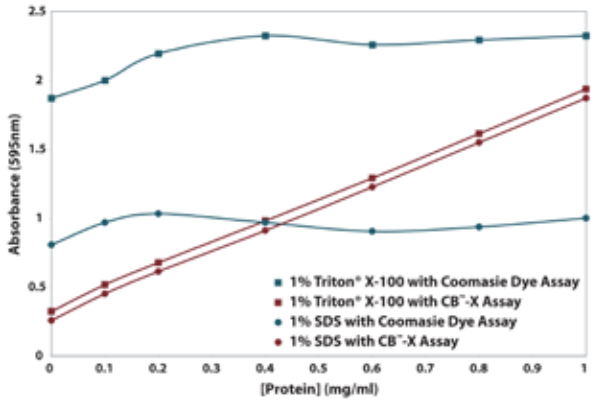

Figure 10: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A linear response to increasing protein concentration was visualized, indicating no interference by the detergents.

Sample Preparation

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

Assay Sensitivity and Sample Size

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

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

Dilute Protein Sample

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

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

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

Time Consideration & Assay Time

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

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

Protein Standards

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

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

Protein-to-Protein Variations

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

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

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

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

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

Instrumentation Requirements

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

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

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

CB-X™

One Assay for All Jobs

A major problem for researchers is to select a protein assay from the vast selection on the market that is compatible with their protein sample.
CB-X™ Protein Assay eliminates this problem as it is designed to be compatible with all commonly used buffers and conditions in protein isolation, storage and assays.

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

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

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

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

If the protein sample does not contain interfering agents then a straightforward single reagent assay is performed to give a linear response. If interfering agents are present or if 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.

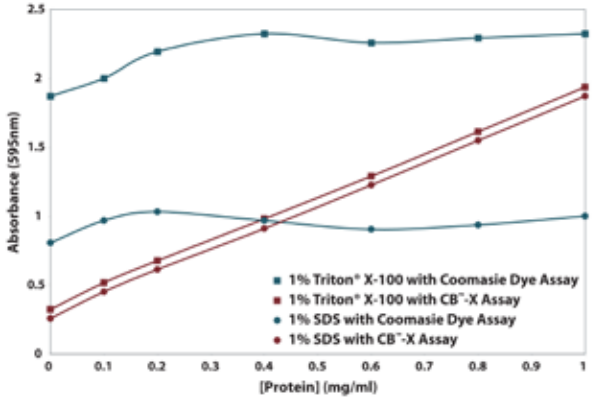

Figure 11: Inhibitory effects of detergents on protein assays are abolished with CB-X™. Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X-100 or 1% SDS were assayed using CB-X™ protein assay. A 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.

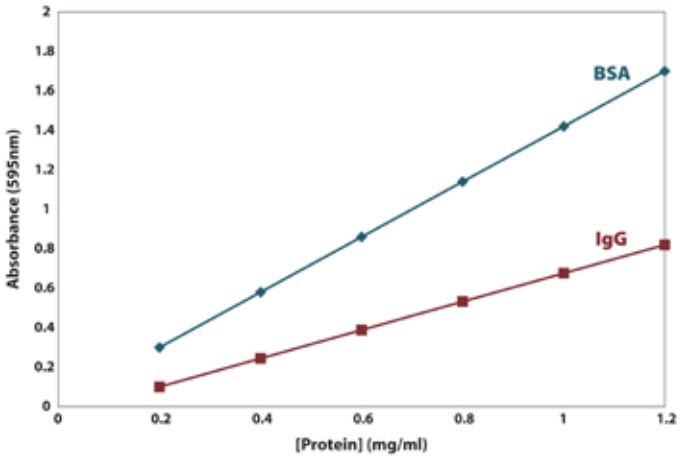

Figure 12: Linear Response and Protein-to-Protein variation with CB™ Protein Assay.

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

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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.

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

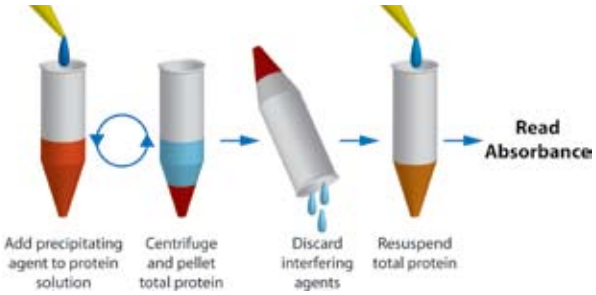

Figure 13: The NI™ Protein Assay Scheme

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.

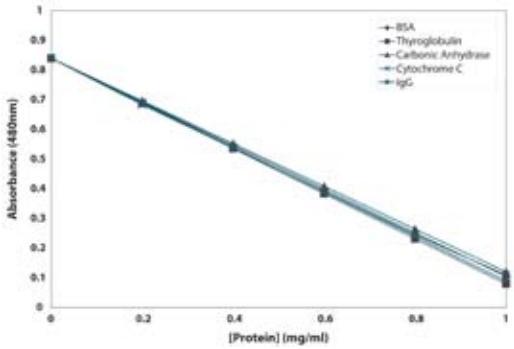

Figure 14: NI™ Protein Assay shows minimal protein-to-protein variation. BSA, thyroglobulin, carbonic anhydrase, cytochrome C and bovine immunoglobulin G were assayed for protein-to-protein variation. The proteins produced identical color and slope in the range of 0.5µg-50µg, giving an average ratio 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 furthered enhanced with Neutralizer™. Neutralizer™ is a unique chemical that sequesters ionic detergents, including SDS, allow the solution to be compatible with the RED 660™ Protein Assay. The Neutralizer™ can also be used with Laemmli loading buffer.

INTERFERENCE TO PROTEIN ASSAY

Agents compatible with the RED 660™ Protein Assay are shown and acceptable concentration of reagents for standard protocols are listed. In most cases, using a correct blank will eliminate or minimize the error caused by interference. * Indicates acceptable concentration when RED 660™ Protein Assay Reagent is supplemented with Neutralizer™.

FEATURES

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

PROTEIN-TO-PROTEIN VARIATION

Protein-dye complex color is primarily the result of binding of the Coomassie dye to the basic and aromatic amino acid residues, especially histidine, arginine and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine; therefore, the RED 660 Protein Assay shows protein-to-protein variations. For greater accuracy, the standard plot should be prepared using a protein sample that has a color response similar to the test sample. Ideally, a pure fraction of the test protein.

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

Table 5: Protein-to-protein variation.

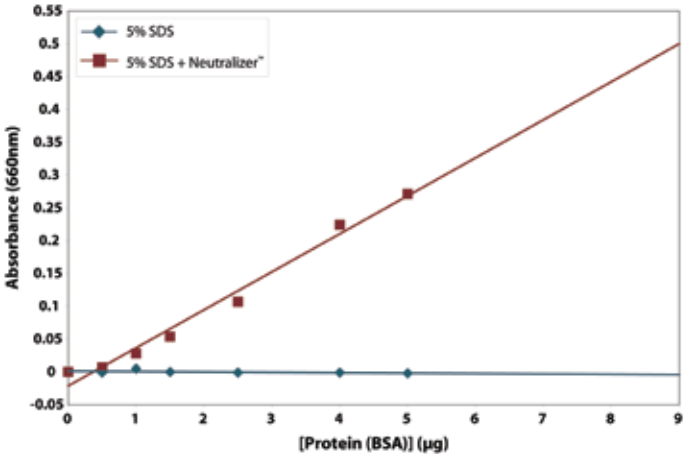

Figure 15: Presence of Neutralizer™ overcomes 5% SDS interference.

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%	Octylthioglucopyranoside	10%
CHAPSO	4%	Octyl-β-glucoside	5%
Citrate	12.5mM	Phenol red	0.5mg/ml
CTAB*	2.5%	PIPES, pH6.8	100mM
Cysteine	350mM	Sodium acetate, pH4.8	100mM
Deoxycholate	0.25%	Sodium chloride	1.25M
DMF	50%	SDS	0.0125%, 5%*
DMSO	50%	Sodium hydroxide	0.125M
DTT	500mM	Sucrose	50%
EDTA	20mM	TCEP	40mM
EGTA	20mM	Thiourea	2M
Ethanol	50%	Tissue PELB™	Dilute 2-fold
FOCUS™ Extraction Buffers	Yes	Tris.HCl, pH8.0	250mM
Glutathione (Reduced)	100mM	Triton® X-100	1%
Glycerol	50%	Triton® X-114	0.5%
Glycine buffer. pH2.8	0.1M	Tween® 20	10%
Guanidine.HCl	2.5M	Urea	8M
HEPES, pH7.5	0.1M		

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

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

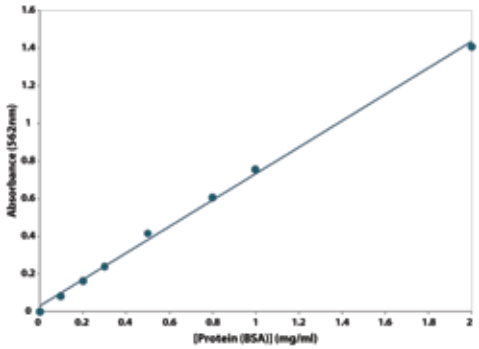

Figure 16: Bicinchoninic Acid (BCA) Protein Assay Standard Curve.

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 ß-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 17: The SPN™ spin columns

SPN™ -HTP PROTEIN ASSAY

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

Figure 18: The SPN™-htp protein assay.

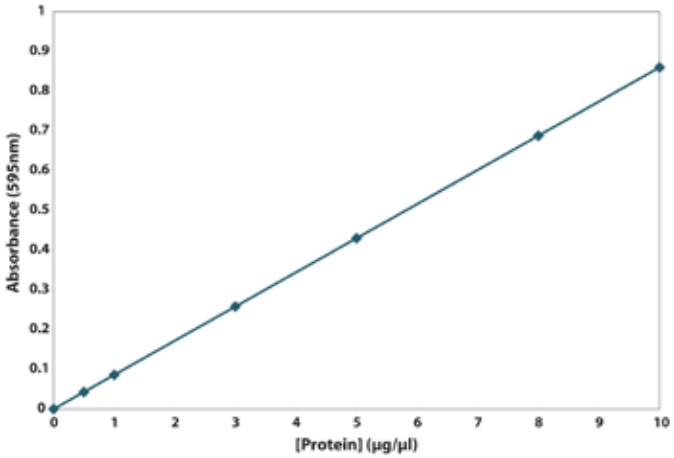

Figure 19: Standard Calibration Plot for SPN™ Protein Assay.

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

Schillace R.V., et al (2009) PLoS ONE 4:e4807

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 dotMETRIC™ Assay

1µl Assay For Rapid Protein Estimation

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

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

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

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

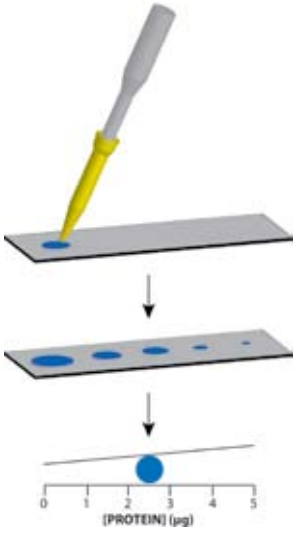

Figure 20: Protein dot METRIC™ Protein Assay Scheme.

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

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

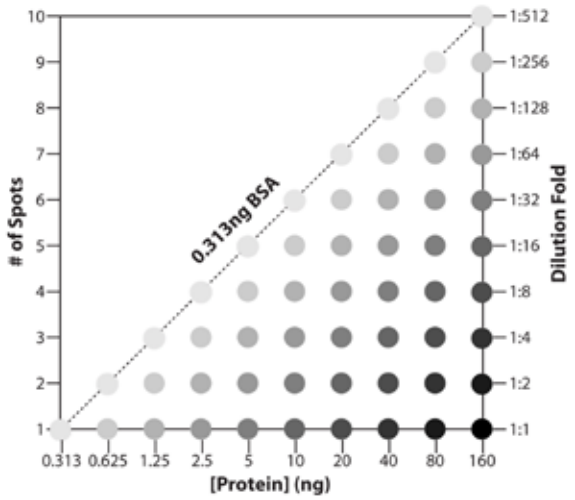

Figure 21: A representation of the DLD™ Protocol.

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

REFERENCES

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Easton, R. et al (1997) J. Neurosci. 17: 9656
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Cat. #	Description	Size
786-20	Protein dotMETRIC™ Kit	>300 Assays
786-21	Protein dotMETRIC™ Kit with Spot Application Device & Glass Capillary Tips	>300 Assays

For further details, visit www.GBiosciences.com

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.

For further details, visit www.GBiosciences.com

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
***dot*METRIC™** (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
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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

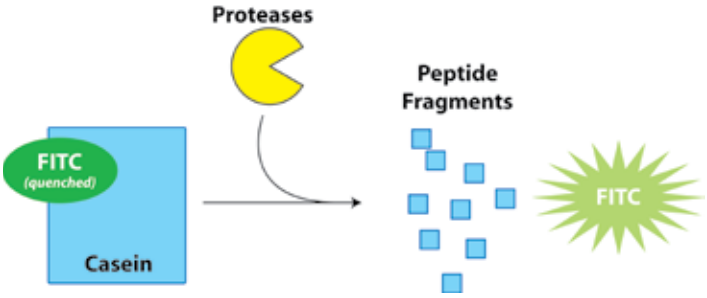

Figure 22: Fluoro™ Protease Assay Scheme

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.

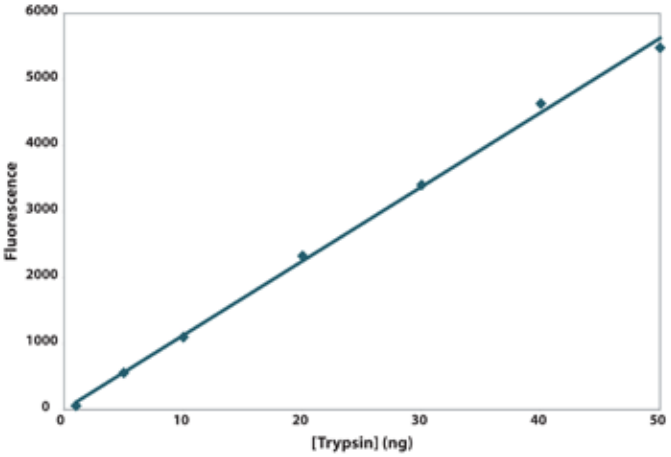

Figure 23: The Linear Response of Fluoro™ Protease Assay

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

For further details, visit www.GBiosciences.com

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.

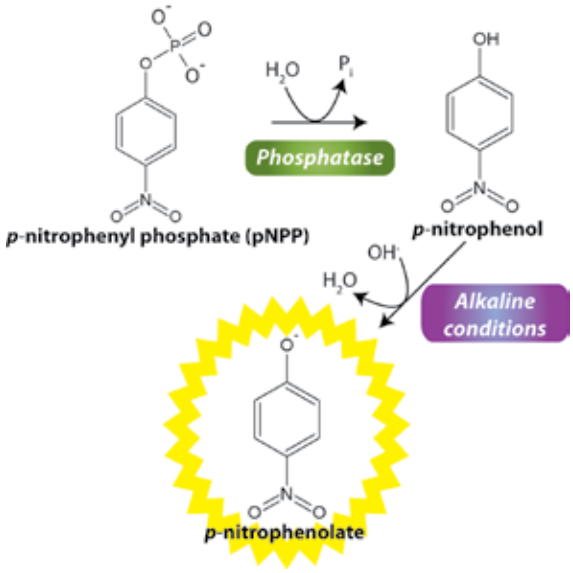

Figure 24: The Phosphatase Assay Scheme

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 stablized solution of sodium fluoride, sodium orthovanadate, sodium pyrophosphate, β-glycerophosphate & sodium molybdate.

REFERENCES

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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-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

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

For further details, visit www.GBiosciences.com

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.

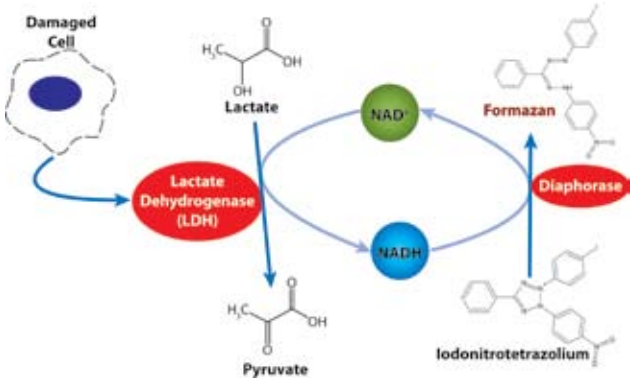

Figure 25: CytoScan™ LDH Cytotoxicity Assay scheme.

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

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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

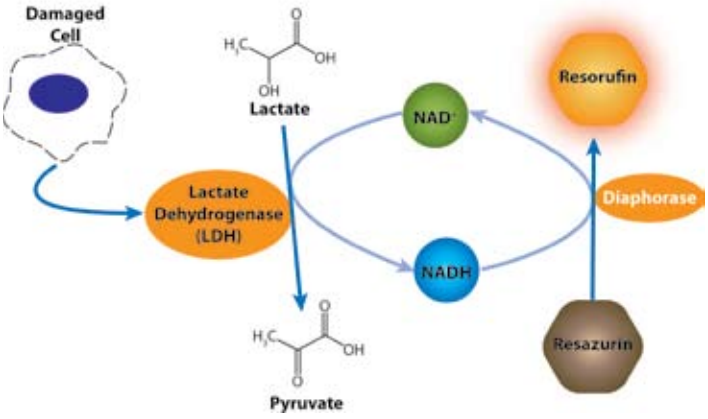

Figure 26: CytoScan™-fluoro cytotoxicity assay scheme

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

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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

For further details, visit www.GBiosciences.com

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.

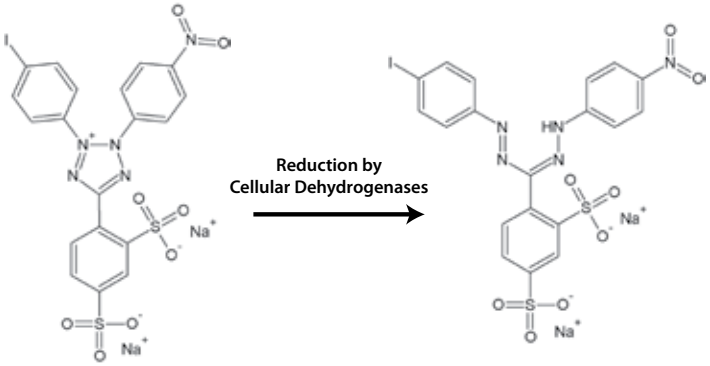

Figure 27: CytoScan™ WST-1 Cell Proliferation assay scheme.

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.

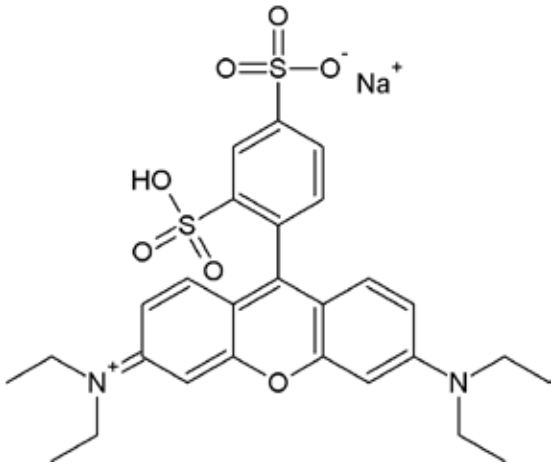

Figure 28: Sulforhodamine B structure.

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

For further details, visit www.GBiosciences.com

ELISAs (Enzyme Linked ImmunoSorbent Assays), or EIAs (Enzyme Immunoassays) are routinely developed and used to detect and quantitate key biochemical molecules, including peptides, proteins, hormones and antibodies. Basically, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a color change in a chemical substrate.

The sensitivity and specificity of ELISA is due to the high specificity of the antibody-antigen interactions.

There are several types of ELISA and the choice of reagents is dependent on the assay used:

- **Direct ELISA:** An antigen is bound directly to the ELISA plate and is detected with a single enzyme labeled antibody.

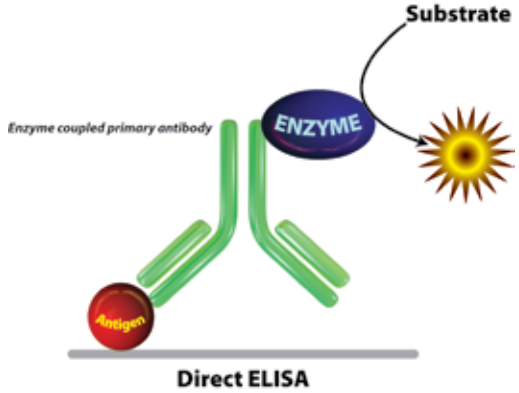

Figure 30: Direct ELISA using a single labeled antibody.

- **Indirect ELISA:** An antigen is bound directly to the ELISA plate, is detected with a primary antibody, which in turn is detected by a enzyme labeled secondary antibody.

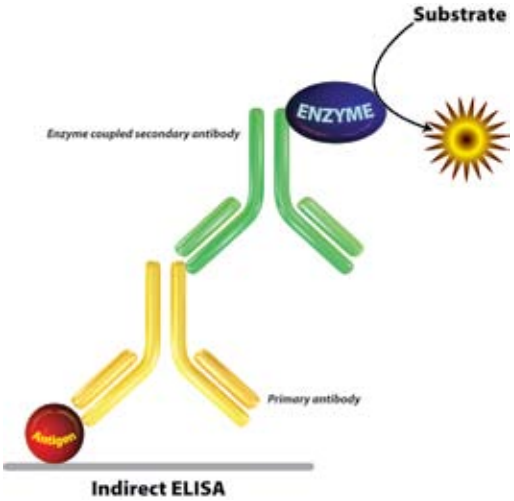

Figure 31: Indirect ELISA using a labeled secondary antibody to detect the primary antibody.

- **Sandwich ELISA:** The most commonly use ELISA format. A primary antibody is bound to the ELISA plate and is used to capture the antigen. The antigen is then detected by a second primary antibody coupled to an enzyme or by a primary antibody followed by an enzyme labeled secondary antibody.

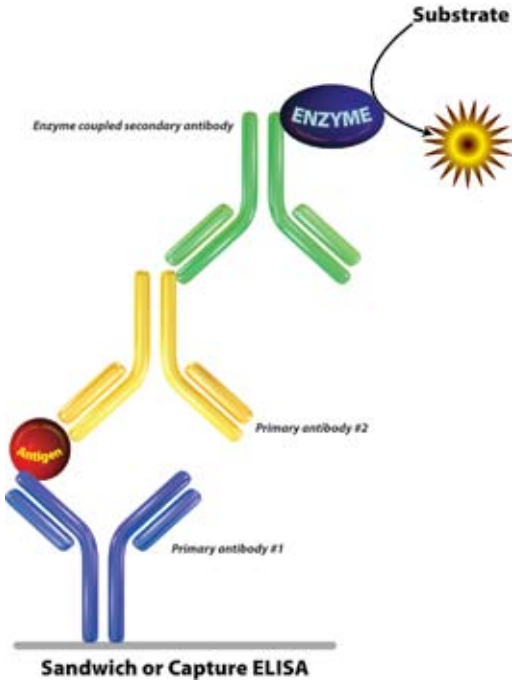

Figure 32: Sandwich ELISA using an antibody to capture the antigen prior to detection.

- **ABC ELISA:** ABC (Avidin-Biotin Complex) ELISA uses a biotin labeled antibody to detect the antigen. The biotin label is detected with a mixture of avidin and biotin labeled enzyme that results in a large complex of avidin, biotin and enzyme. This amplifies the signal from each antigen, compared to the above ELISA methods.

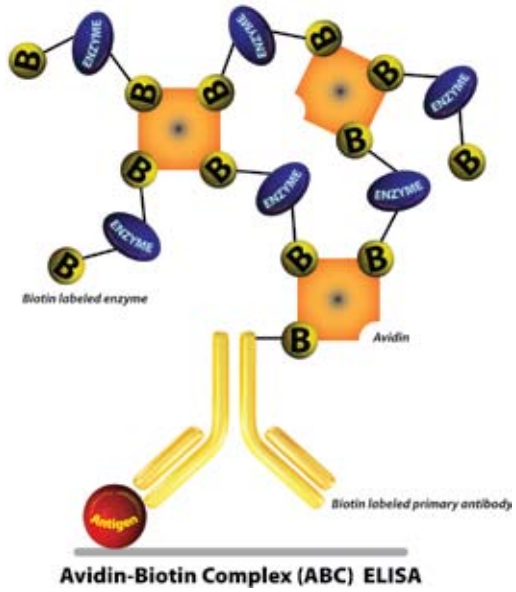

Figure 33: ABC ELISA using an avidn-biotin complex to enhance the ELISA signal.

Well-Coated™ plates are available as single 96-well plates or as 12 x 8-well strips in a 96-well holder. The plates are supplied as clear, white and black plates for colorimetric, chemiluminescence and fluorescent detection systems respectively.

FOR ANTIBODY BINDING

Well-Coated™ Protein A, Protein G & Protein A/G

Bind constant (Fc) domain of antibodies

Designed to bind the constant (Fc) region of immunoglobulins ensuring that the antigen binding domain of the antibody is orientated away from the plate, offering maximum exposure of the binding site. Protein A-G contains 4 binding sites from protein A and 2 from protein G offering maximum range of specificity and binding capacity. The immunoglobulin orientation improves the antibody capacity compared to plates that are coated directly with antibodies.

The plates are for single antibody assays and are not suitable for multiple assays (sandwich ELISAs) as the first antibody will not block all IgG binding sites and therefore false positives will occur with the second antibody. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are available.

See table for antibody binding affinities of Protein A, Protein G and Protein A/G

FEATURES

- Protein A/G has highest specificity and capacity
- Retains antibody activity & orients antibody for maximum binding
- Reduce non-specific binding
- Binds ~4pmol rabbit IgG/well

Cat. #	Description	Size
786-731	Well-Coated™ Protein A, 8-well strip plate, Clear	5 Plates
786-770	Well-Coated™ Protein A, 96 well plate, Black	5 Plates
786-730	Well-Coated™ Protein A, 96 well plate, Clear	5 Plates
786-771	Well-Coated™ Protein A, 96 well plate, White	5 Plates
786-733	Well-Coated™ Protein G, 8-well strip plate, Clear	5 Plates
786-774	Well-Coated™ Protein G, 96 well plate, Black	5 Plates
786-732	Well-Coated™ Protein G, 96 well plate, Clear	5 Plates
786-775	Well-Coated™ Protein G, 96 well plate, White	5 Plates
786-735	Well-Coated™ Protein A/G, 8-well strip plate, Clear	5 Plates
786-772	Well-Coated™ Protein A/G, 96 well plate, Black	5 Plates
786-734	Well-Coated™ Protein A/G, 96 well plate, Clear	5 Plates
786-773	Well-Coated™ Protein A/G, 96 well plate, White	5 Plates

Well-Coated™ Protein L

Bind kappa light chains of immunoglobulins

Designed to bind the kappa light chains of immunoglobulins without interfering with the antigen binding site. Well-Coated™ Protein L plates bind a greater range of immunoglobulin classes and subclasses compared to Protein A, G and A/G. Protein L will bind to all classes of IgG, including IgG, IgM, IgA, IgE and IgD, and binds to single chain variable fragments (scFv and Fab fragments).

The plates are for single antibody assays and are not suitable for multiple assays (sandwich ELISAs) as the first antibody will not block all IgG binding sites. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Retains antibody activity
- Binds to all classes of IgG, including IgG, IgM, IgA, IgE and IgD
- Reduced non-specific binding as plates are pre-blocked

TECHNICAL INFORMATION

- Only binds kappa I, III and IV in human and kappa I in mouse
- May be specific for certain kappa subgroups in other species
- Binds scFv without interfering with antigen binding
- Has weak binding affinity for rabbit immunoglobulins
- No binding affinity for bovine, goat or sheep immunoglobulins
- No binding affinity for lambda light chains

Cat. #	Description	Size
786-737	Well-Coated™ Protein L, 8-well strip plate, Clear	5 Plates
786-776	Well-Coated™ Protein L, 96 well plate, Black	5 Plates
786-736	Well-Coated™ Protein L, 96 well plate, Clear	5 Plates
786-777	Well-Coated™ Protein L, 96 well plate, White	5 Plates

Species	Antibody Class	Protein A	Protein G	Protein A/G
Mouse	Total IgG	*****	*****	*****
	IgM	-	-	-
	IgG_1	*	***	***
	IgG_{2a}	*****	*****	*****
	IgG_{2b}	*****	*****	*****
	IgG_3	*****	*****	*****
Human	Total IgG	*****	*****	*****
	IgG_1	*****	*****	*****
	IgG_2	*****	*****	*****
	IgG_3	*	*****	*****
	IgG_4	*****	*****	*****
	IgM	*	-	*
	IgD	-	-	-
	IgA	*	-	*
	Fab	*	*	*
	ScFv	*	-	*
Rat	Total IgG	*	***	***
	IgG_1	*	***	***
	IgG_{2a}	-	*****	*****
	IgG_{2b}	-	*	*
	IgG_{2c}	*****	*****	*****
Rabbit	Total IgG	*****	*****	*****
Goat	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****
Cat	Total IgG	*****	*	*****
Chicken	Total IgY	-	-	-
Cow	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****
Dog	Total IgG	*****	*	*****
Guinea Pig	Total IgG	*****	*	*****
Horse	Total IgG	*	*****	*****
	IgG(ab)	*	-	*
	IgG(c)	*	-	*
	IgG(T)	-	*****	*****
Pig	Total IgG	*****	*	*****
Sheep	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****

Table 11: Relative affinity of Protein A, Protein G and Protein A/G for immunoglobulins.

Well-Coated™ Antibody

Bind mouse or rabbit IgG antibodies

Designed to specifically bind either mouse or rabbit IgG making them suitable for binding assays using low quantities of antibodies or antibodies that denature on direct binding to polystyrene plates. Another advantage is that the specificity to IgG means purified antibodies are not essential.

Suitable for direct, indirect, competitive and sandwich assays. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Binds ~7pmol mouse IgG/well or ~12pmol rabbit IgG/well
- Prevents denaturation of antibodies unlike direct binding
- Species specific binding

Cat. #	Description	Size
786-739	Well-Coated™ Antibody (goat α-mouse), 8-well strip, Clear	5 Plates
786-758	Well-Coated™ Antibody (goat α-mouse), 96 well, Black	5 Plates
786-738	Well-Coated™ Antibody (goat α-mouse), 96 well, Clear	5 Plates
786-759	Well-Coated™ Antibody (goat α-mouse), 96 well, White	5 Plates
786-741	Well-Coated™ Antibody (goat α-rabbit), 8-well strip, Clear	5 Plates
786-760	Well-Coated™ Antibody (goat α-rabbit), 96 well, Black	5 Plates
786-740	Well-Coated™ Antibody (goat α-rabbit), 96 well, Clear	5 Plates
786-761	Well-Coated™ Antibody (goat α-rabbit), 96 well, White	5 Plates

FOR BIOTIN BINDING

Well-Coated™ Neutravidin™

Bind biotinylated molecules & proteins

Designed to specifically bind biotinylated molecules, including biotin tagged antibodies, with minimal non-specific binding. This is particularly advantageous for antibodies known to denature upon direct binding to polystyrene plates.

Neutravidin™ is in many respects similar to avidin and streptavidin except that it has no carbohydrate side chains to eliminate lectin binding; is of near neutral pI (6.3) to reduce non-specific adsorption; lacks the RYD sequence eliminating interaction with RGD domain of adhesion receptors. The binding of Neutravidin™ is similar to that of avidin and streptavidin with less non-specific binding.

Suitable for direct, indirect, competitive and sandwich assays. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Binding capacity: ~15pmol D-biotin/well
- High binding affinity for biotin
- Low non-specific binding
- Reduced non-specific binding as plates are pre-blocked

Cat. #	Description	Size
786-743	Well-Coated™ Neutravidin™, 8-well strip plate, Clear	5 Plates
786-766	Well-Coated™ Neutravidin™, 96 well plate, Black	5 Plates
786-742	Well-Coated™ Neutravidin™, 96 well plate, Clear	5 Plates
786-767	Well-Coated™ Neutravidin™, 96 well plate, White	5 Plates

Well-Coated™ Streptavidin

Bind biotinylated molecules & proteins

Designed to specifically bind biotinylated molecules, including biotin tagged antibodies. This is particularly advantageous for antibodies known to denature upon direct binding to polystyrene plates.

Biotin exhibits an extraordinary binding affinity for streptavidin ($Ka=10^{15}M^{-1}$). Biotin and streptavidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-streptavidin bonds can only be reversed by denaturing the streptavidin with 8M guanidine-hydrochloride at pH1.5 or by autoclaving. Streptavidin has no carbohydrate and its solubility (isoelectric pH5) in aqueous buffer and the level of non-specific binding is lower than avidin, due to the lack of carbohydrate groups.

Suitable for direct, indirect, competitive and sandwich assays. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Binding capacity: ~5pmol D-biotin/well
- High binding affinity for biotin
- Low non-specific binding
- Ideal for peptides, antibodies and small hydrophilic molecules
- Reduced non-specific binding as plates are pre-blocked

Cat. #	Description	Size
786-745	Well-Coated™ Streptavidin, 8-well strip plate, Clear	5 Plates
786-778	Well-Coated™ Streptavidin, 96 well plate, Black	5 Plates
786-744	Well-Coated™ Streptavidin, 96 well plate, Clear	5 Plates
786-779	Well-Coated™ Streptavidin, 96 well plate, White	5 Plates

Well-Coated™ Biotin

Bind avidin, streptavidin or Neutravidin™ conjugated molecules

Designed to specifically bind avidin, streptavidin or Neutravidin™ conjugated molecules, including enzyme conjugates.

Biotin exhibits an extraordinary binding affinity for avidin ($Ka=10^{15}M^{-1}$) and streptavidin ($Ka=10^{15}M^{-1}$). Biotin and avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by autoclaving. Streptavidin and Neutravidin™ in many respects are similar to avidin except that they have no carbohydrate and their solubility in aqueous buffer is much lower than avidin. Neutravidin™ also lacks the RYD sequence eliminating interaction with RGD domain of adhesion receptors. The binding of streptavidin and Neutravidin™ is similar to that of avidin, but with less non-specific binding.

The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are available.

FEATURES

- Binds avidin, streptavidin and Neutravidin™ conjugated molecules
- Reduced non-specific binding as plates are pre-blocked

Cat. #	Description	Size
786-747	Well-Coated™ Biotin, 8-well strip plate, Clear	5 Plates
786-762	Well-Coated™ Biotin, 96 well plate, Black	5 Plates
786-746	Well-Coated™ Biotin, 96 well plate, Clear	5 Plates
786-763	Well-Coated™ Biotin, 96 well plate, White	5 Plates

FOR PROTEIN/PEPTIDE BINDING

Well-Coated™ Nickel

Bind 6X His-tagged proteins

Designed to specifically bind 6X histidine (polyhistidine) tagged proteins and peptides. The plates isolate polyhistidine-tagged proteins direct from bacterial lysates for subsequent ELISA protocols. The wells are coated to a 200µl depth and are supplied pre-blocked. Clear, white and black plates are available.

FEATURES

- Binding Capacity: ~9mol His-tagged protein/ well
- Low non-specific binding
- Ideal for proteins and peptides with polyhistidine (6X His) tag

Cat. #	Description	Size
786-749	Well-Coated™ Nickel, 8 well strip plate, Clear	5 Plates
786-768	Well-Coated™ Nickel, 96 well plate, Black	5 Plates
786-748	Well-Coated™ Nickel, 96 well plate, Clear	5 Plates
786-769	Well-Coated™ Nickel, 96 well plate, White	5 Plates

Well-Coated™ Glutathione

Bind GST-tagged proteins

Designed to specifically bind GST (Glutathione S-Transferase) tagged proteins and peptides. The plates have immobilized glutathione and isolate GST-tagged proteins direct from bacterial lysates for subsequent ELISA protocols. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are available.

FEATURES

- Binding Capacity: ~9mol purified GST/ well
- Low non-specific binding

Cat. #	Description	Size
786-751	Well-Coated™ Glutathione, 8-well strip plate, Clear	5 Plates
786-764	Well-Coated™ Glutathione, 96 well plate, Black	5 Plates
786-750	Well-Coated™ Glutathione, 96 well plate, Clear	5 Plates
786-765	Well-Coated™ Glutathione, 96 well plate, White	5 Plates

Well-Coated™ Amine Binding

Bind primary amines of peptides & proteins

Designed to specifically bind primary amines of peptides, proteins and other molecules and overcome the inherent issues of passive adsorption for immobilizing peptides and other ligands for binding assays.

Well-Coated™ Amine Binding plates are maleic anhydride activated plates that react with primary amines to form amide bonds that are stable at pH≥7. Acidic conditions will hydrolyze the bonds releasing the peptide/ligand, therefore binding of peptide/ligand to plates should be performed at pH8-9 and the binding assays or ELISA should be performed at pH≥7.

The wells are coated to a 200µl depth and are supplied pre-blocked. Clear, white and black plates are available.

FEATURES

- Binding capacity: ~120pmol HOOK™ Biotin Pentylamine/well
- Rapid binding of primary amines
- Stable plates
- Reduced non-specific binding as plates are pre-blocked

Cat. #	Description	Size
786-753	Well-Coated™ Amine Binding, 8 well strip plate, Clear	5 Plates
786-756	Well-Coated™ Amine Binding, 96 well plate, Black	5 Plates
786-752	Well-Coated™ Amine Binding, 96 well plate, Clear	5 Plates
786-757	Well-Coated™ Amine Binding, 96 well plate, White	5 Plates

Well-Coated™ Sulfhydryl Binding

Bind free sulfhydryls of peptides & proteins

Designed to specifically bind free sulfhydryls of peptides, proteins and other molecules and overcome the inherent issues of passive adsorption for immobilizing peptides and other ligands for binding assays.

Well-Coated™ Sulfhydryl Binding plates are maleimide activated plates that react with free sulfhydryls to form stable thioether bonds at pH 6.5-7.5. pH >7.5 significantly increases the reaction of amines with the maleimide groups.

The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are available.

FEATURES

- Binding capacity: ~120pmol sulfhydryl peptide/well
- Rapid binding of sulfhydryls
- Reduced non-specific binding as plates are pre-blocked

Cat. #	Description	Size
786-755	Well-Coated™ Sulfhydryl Binding, 8 well strip plate, Clear	5 Plates
786-780	Well-Coated™ Sulfhydryl Binding, 96 well plate, Black	5 Plates
786-754	Well-Coated™ Sulfhydryl Binding, 96 well plate, Clear	5 Plates
786-781	Well-Coated™ Sulfhydryl Binding, 96 well plate, White	5 Plates

NON-ANIMAL BLOCKING AGENTS

A major drawback of animal protein blocking solutions, such as BSA, casein and milk powders, is they are derived from animal sources. The presence of animal proteins can often lead to high non-specific backgrounds as antigens and antibodies, generated in animals, interact with the "blocking" animal proteins.

NAP-BLOCKER™

Non-animal blocking protein preparation

For improved assay sensitivity, minimal non-specific binding, and a high signal-to-background ratio. NAP-BLOCKER™ ensures no cross-reaction with your animal source antigens and antibodies, due to being 100% free of animal proteins. NAP-BLOCKER™ is easy to use and generates high publication quality blots (Figure 132).

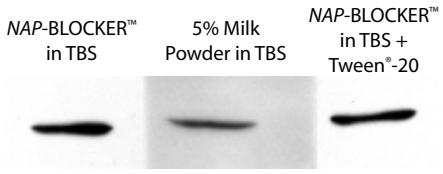

Figure 34: Comparison of NAP-BLOCKER™ and milk powder. Protein lysates were transferred to PVDF membranes and blocked for 90 minutes as indicated. The membranes were probed for actin and subsequently exposed to film for 20 minutes.

NAP-BLOCKER™ is free from biotin and other cross-reacting agents present in most of the animal source blocking agents. NAP-BLOCKER™ ensures uniform blocking without non-specific binding. It is simple to use with improved results compared to milk powder preparations.

NAP-BLOCKER™ is supplied as a pre-made [2X] solution; simply dilute with any buffer and block nitrocellulose or PVDF membranes. Alternatively, NAP-BLOCKER™ is supplied in PBS or TBS buffers.

FEATURES

- Non-animal protein blocking agent
- 2X concentrated solution
- Uniform blocking with reduced background staining

APPLICATIONS

- For Western blots, dot blots, ELISA and assay development

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Cat. #	Description	Size
786-190	NAP-BLOCKER™ [2X]	2 x 500ml
786-190P	NAP-BLOCKER™ in PBS [2X]	2 x 500ml
786-190T	NAP-BLOCKER™ in TBS [2X]	2 x 500ml

Protein-Free™

Eliminates protein related cross-reactivity

Protein-Free Blocking Buffer does not contain protein; it is a proprietary formulation of non-protein agents that eliminates non-specific binding sites in ELISA, blotting, immunohistochemistry and other applications. The absence of protein eliminates problems associated with traditional protein based blockers, such as cross-reactivity and interference from glycosylated proteins.

Eliminates any concern associated with regulatory compliance issues where use of animal source components are restricted. Furthermore, Protein-Free™ Blocking Buffer is compatible with antibodies and avidin/biotin based systems and results in high signal to background ratios.

For user's convenience Protein-Free Blocking Buffers are supplied in widely used TBS (Tris-buffered saline at pH 7.5) and PBS buffers (phosphate-buffered saline at pH 7.5) as well as in separate formulations containing Tween® 20 for improving blocking efficiencies.

FEATURES

- Protein free blocking agent
- Eliminate cross reactivity with animal source antibodies
- High signal to background ratios
- Four convenient formats, with and without detergent
- Ready-to-use

APPLICATIONS

- Suitable for Western blot and ELISA applications

Cat. #	Description	Size
786-664	Protein-Free Blocking Buffer-PBS	500ml
786-665	Protein-Free Blocking Buffer-PBST	500ml
786-662	Protein-Free Blocking Buffer-TBS	500ml
786-663	Protein-Free Blocking Buffer-TBST	500ml

NON-ANIMAL SERA PROTEIN BLOCKING AGENTS

FISH-Blocker™

Uses fish proteins to eliminate cross reactivity

FISH-Blocker™ is a blocking agent that uses a fish protein as the primary blocking agent. The use of a fish protein, a non-mammalian protein, eliminates or minimizes the interaction of antibodies raised in mammals. FISH-Blocker™ is one of the best blocking agents for immunoassays and it offers an alternative to milk-based blocking agents, minimizing the risk of non-specific binding of antibodies during the immunodetection process and lowering the background.

FEATURES

- A non mammailan protein to elimate non-specific binding
- High signal to background ratio
- Ready-to-use

APPLICATIONS

- Suitable for Western blot and ELISA applications

Cat. #	Description	Size
786-675	FISH-Blocker™ in PBS	500ml
786-674	FISH-Blocker™ in TBS	500ml

Superior™ Blocking Buffer

An enhanced blocker in multiple formats

Superior™ Blocking Buffer contains a proprietary antigenically non-determinant protein for blocking non-specific sites during ELISA, membrane blotting, immunohistochemistry and other applications.

Superior™ Blocking Buffer is ideal for a high signal to background ratio in most system. Superior™ Blocking Buffer uses a non-serum protein and does not contain biotin or other animal source proteins to interfere with immuno-complexes. Superior™ Blocking Buffer is suitable for assays that use avidin/streptavidin systems.

Available in multiple formats using TBS, PBS, TBS with 0.05% Tween® 20 or PBS with 0.05% Tween® 20. Also supplied as a convenient dry form that is stable at room temperature. Each dry format pack makes 200ml Superior™ Blocking Buffer.

FEATURES

- Non serum protein blocking agent
- Rapid blocking times; ~2 minutes for ELISA

Cat. #	Description	Size
786-660	Superior™ Blocking Buffer in PBS	500ml
786-661	Superior™ Blocking Buffer in PBST	500ml
786-658	Superior™ Blocking Buffer in TBS	500ml
786-659	Superior™ Blocking Buffer in TBST	500ml
786-601	Superior Blocking Buffer-Dry Blend in PBS	5 Packs
786-657	Superior™ Blocking Buffer-Dry Blend in TBS	5 Packs

FirstChoice™

Ideal for new assay development

A proprietary protein formulation that offers greater versatility and lack of cross-reactivity. FirstChoice™ Blocking Buffer is ideal as a first choice for optimization of new assays, systems or when determining the optimal blocking buffer for elimination of non-specific binding sites in ELISA, blotting, immunohistochemistry and other applications. FirstChoice™ Blocking Buffers are compatible with antibodies and avidin/biotin based systems and results in high signal to background.

For users convenience FirstChoice™ Blocking Buffers are supplied in widely used TBS (Tris-buffered saline at pH 7.5) and PBS (phosphate-buffered saline at pH 7.5) buffers as well as in separate formulations containing Tween® 20 for improving blocking efficiencies.

FEATURES

- Ready-to-use
- Available as TBS or PBS with optional Tween® 20
- Animal serum & Biotin free
- Ideal blocking buffer for setting up new assays and systems

Cat. #	Description	Size
786-668	FirstChoice™ Blocking Buffer-PBS	500ml
786-669	FirstChoice™ Blocking Buffer-PBST	500ml
786-666	FirstChoice™ Blocking Buffer-TBS	500ml
786-667	FirstChoice™ Blocking Buffer-TBST	500ml

BLOK™ BLOTTO

A 5% modified milk protein blocking solution

FEATURES

- Ready-to-use 5% novel modified milk protein solution
- Use for Westerns, ELISA, and dot blot blocking
- Blocking in 10-15 minutes

Cat. #	Description	Size
786-192	BLOK™ BLOTTO 5% non fat milk solution	2 x 500ml

BLOT-QuickBlocker™

A modified milk protein blocking agent

BLOT-QuickBlocker™ is a novel modified milk protein that is highly soluble and does not inhibit peroxidase detection. The modified milk protein has high blocking efficiency with a clear background.

FEATURES

- Readily soluble and produces semi-clear solution
- No inhibition to peroxidase
- Produces clear background
- Higher blocking efficiency
- Blocking time 30-60 minutes
- Fat free

APPLICATIONS

- For Western blots and dot blots

REFERENCES

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Malloy, P. and Feldman, D. (2009) Endocrinology 150: 679
Kroemer, J.A and Webb, B.A. (2006) J Virol. 80: 12219
Benou, C. et al (2005) J. Immunology. 174: 5407
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Kroemer, J.A and Webb, B.A. (2005) J Virol. 79: 7617
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, Q. et al (2004) Reproduction 128: 555
Alvarez, G. R. et al (2003) J. Immunol. 171: 6766

Cat. #	Description	Size
786-011	BLOT-QuickBlocker™	175g

BLOK™ Casein

A 1% casein protein blocking solution

FEATURES

- Ready-to-use
- Available in your choice of TBS or PBS buffers.

Cat. #	Description	Size
786-194	BLOK™ Casein in PBS, 1% solution	2 x 500ml
786-196	BLOK™ Casein in TBS, 1% solution	2 x 500ml

PROTEIN BLOCKING AGENTS

BLOK™ BSA

A 10% BSA protein blocking solution

- For blocking Westerns, ELISA and dot Blots
- Ready-to-use
- Available in your choice of TBS or PBS buffers

Cat. #	Description	Size
786-193	BLOK™ BSA in TBS, 10% solution	125ml
786-195	BLOK™ BSA in PBS, 10% solution	125ml

PROTEOMIC GRADE DETERGENTS

10% solutions of ultra low carbonyl & peroxide contaminants

Many commercial grade detergents contain elevated levels of sulfhydryl oxidizing agents, peroxides, salts and carbonyl compounds. The proteins that are isolated with these detergents are highly susceptible to contaminating peroxides and carbonyls. The peroxides will oxidize proteins and the carbonyl groups will form Schiff's bases with the proteins that will interfere with a protein's structure.

Our Proteomic Grade Detergent Solutions contain reduced peroxides and carbonyl compounds. In addition, the detergents have less than 50µS conductivity. These detergents are offered as 10% aqueous solutions, sealed under inert gas and are suitable for protein applications. These non-ionic detergents are suitable for isolating membrane-protein complexes.

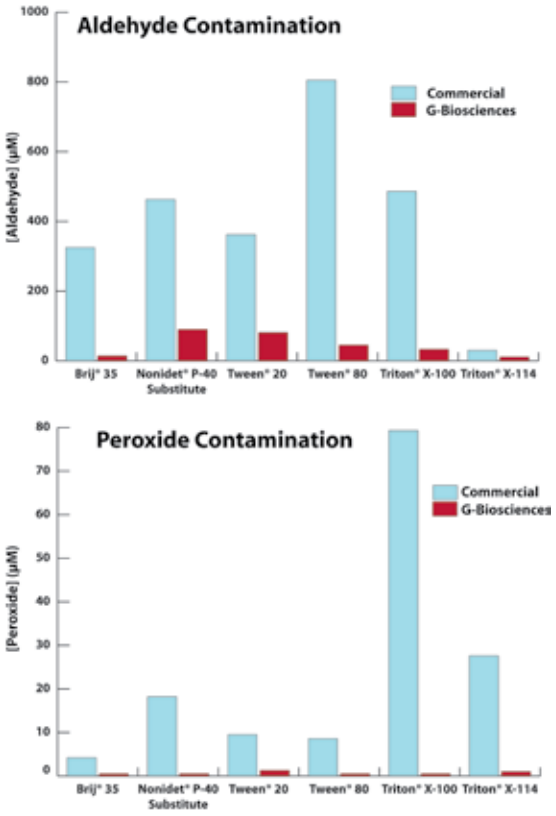


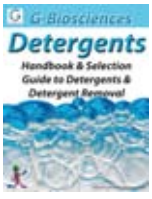
Figure 35: Comparison of aldehyde (top) and peroxide (bottom) concentration in G-Biosciences Proteomic Grade Detergent Solutions and non-proteomic grade commercially available detergents.

FEATURES

- Low peroxide contamination
- Low carbonyl contamination
- Low conductivity
- Reduced metal ions
- 10% aqueous solutions
- Sealed under inert gas to prevent oxidation

We offer a selection of widely used Proteomic Grade Detergent Solutions. The aldehyde and peroxide levels are <100µM and <50µM respectively with a conductivity of <50µS.

A large selection of reserach grade detergents are also available, see the Detergent technical handbook or visit www.GBiosciences.com for more details.



PHOSPHATE BUFFER SALINE (PBS)

femtoPBST™ Wash Buffer

10X concentrated PBS wash buffers to enhance sensitivity of your immunoassays by minimizing "washing out" of antibodies. Wash buffer contains Tween® 20 for improved washing.

10X PBS

10X concentrated PBS solutions for the use as wash buffers for Western blotting, ELISA and other applications.

Dry Buffer Packs

Just add water to generate ready-to-use buffers

- **JAW™ Phosphate Buffered Saline (PBS) [1X]** packs make 1L of 2.7mM potassium chloride, 127mM sodium chloride and 10mM phosphate buffer (pH 7.3-7.5)
- **JAW™ Phosphate Buffered Saline (PBS) [10X]** packs make 1L of 27mM potassium chloride, 1.37M sodium chloride and 0.1M phosphate buffer (pH7.3-7.5)

Cat. #	Description	Size
786-162	*femto* PBST™ [10X]	250ml
786-027	PBS [10X]	500ml
R027	PBS [10X]	1L
R028	PBS [10X]	1gal
786-289	JAW™ Phosphate Buffered Saline [1X] (1L/ pack)	20 packs
RC-147	JAW™ Phosphate Buffered Saline [10X] (10L/pack)	2 packs

TRIS BUFFERED SALINE (TBS)

femtoTBST™ Wash Buffer

10X concentrated TBS wash buffers to enhance sensitivity of your immunoassays by minimizing "washing out" of antibodies.Wash buffer contains Tween® 20 for improved washing.

10X TBS

10X concentrated TBS solutions for the use as wash buffers for Western blotting, ELISA and other applications.

Dry Buffer Packs

Just add water to generate ready-to-use buffers

- **JAW™ Tris Buffered Saline [1X]** packs make 1L of 25mM Tris, 140mM sodium chloride, 3mM potassium chloride, pH 7.25-7.55
- **JAW™ Tris Buffered Saline (TBS) [20X]** packs make 1L of 0.5M Tris, 2.8M sodium chloride, 60mM potassium chloride, pH 7.25-7.55

Cat. #	Description	Size
786-161	*femto* TBST™ [10X]	250ml
R029	TBS [10X])	1L
R030	TBS [10X])	1gal
786-288	JAW™ Tris Buffered Saline [1X] (1L/ pack)	20 packs
RC-148	JAW™ Tris Buffered Saline [20X] (20L/pack)	1 packs

ENZYME CONJUGATED SECONDARY ANTIBODIES

Horseradish Peroxidase (HRP) Conjugated

Horseradish peroxidase is a 44kDa glycoprotein with 4 lysine residues for conjugation to a labelled molecule. It produces a colored, fluorimetric or luminescent derivative of the labeled molecule allowing it to be detected and quantified. Horseradish peroxidase is ideal in many respects for secondary antibody conjugation because it is smaller, more stable and less expensive than other popular alternatives such as alkaline phosphatase. It also has a high turnover rate that allows generation of strong signals in a relatively short time span. The activity of the HRP enzyme is inhibited by cyanides, azides and sulfides.

Secondary antibodies conjugated to horseradish peroxidase (HRP) are isolated from antisera by immunoaffinity chromatography using antigen coupled to sepharose beads. Supplied lyophilized from 0.01M sodium phosphate, 0.25M NaCl, pH7.1 with 15mg/ml BSA and 0.01% thimerosal.

- Western blotting/ Immunoblotting: 1:5,000-1:100,000
- ELISA: 1:5,000-1:100,000
- Immunohistochemistry: 1:500-1:5,000

Cat. #	Description	Size
786-R41	Horseradish peroxidase (HRP) labeled goat α-human IgG	2ml
786-R38	HRP labeled goat α-mouse IgG	2ml
786-R39	HRP labeled goat α-rabbit IgG	2ml
786-R40	HRP labeled goat α-rat IgG	2ml
786-R42	HRP labeled rabbit α-goat IgG	1.5ml
786-R48	HRP labeled rabbit α-human IgG	1.5ml

Alkaline Phosphatase (AP) Conjugated

Alkaline phosphatase is a large 140kDa protein that hydrolyzes phosphate groups from substrates, resulting in a colored, fluorimetric or luminescent derivative.

Secondary antibodies conjugated to horseradish peroxidase (HRP) are isolated from antisera by immunoaffinity chromatography using antigen coupled to sepharose beads. Supplied lyophilized from 0.01M sodium phosphate, 0.25M NaCl, pH7.1 with 15mg/ml BSA and 0.01% thimerosal.

- Western blotting/ Immunoblotting: 1:5,000-1:100,000
- ELISA: 1:5,000-1:100,000
- Immunohistochemistry: 1:500-1:5,000

Cat. #	Description	Size
786-R46	Alkaline phosphatase (AP) labeled goat α-human IgG	1ml
786-R43	AP labeled goat α-mouse IgG	1ml
786-R44	AP labeled goat α-rabbit IgG	1ml
786-R45	AP labeled goat α-rat IgG	1ml
786-R47	AP labeled rabbit α-goat IgG	1ml
786-R49	AP labeled rabbit α-human IgG	1ml

CITED REFERENCES FOR SECONDARY ANTIBODIES

Li, Q. et al (2006) Reproduction 131:553
Benou, C. et al (2005) J. Immunol. 174: 5407
Wang, Y. et al (2005) J. Immunol. 174: 5687
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, Q. et al (2004) Reproduction 128: 555
Alvarez, G.R. et al (2003) J. Immunol. 171: 6766

femtoELISA™

Complete ELISA kits for detection of horseradish peroxidase or alkaline phosphatase

Although the principle of ELISA is very simple, the optimization and perfection of the assay is not. FemtoELISA™ contains all the crucial reagents necessary for a successful ELISA, including an enhanced blocking agent, washing buffer and an ultra sensitive colorimetric enzyme substrate.

femto-ELISA™ kits utilize a non-animal protein blocker, NAP-BLOCKER™ that minimizes cross-reactivity with researcher's antigens and antibodies.

For HRP detection, an improved, ultra sensitive, non-volatile, stable colorimetric substrate based on tetramethyl benzidine (TMB). femtoELISA™-HRP substrate does not require hydrogen peroxide that can have detrimental effects on assays.

For the detection of alkaline phosphatase, a pNPP (p-nitrophenylphosphate) based substrate with superior stability compared to commonly used pNPP tablets and solutions is offered. The improved stability ensures minimal background absorbance over longer periods compared to normal pNPP substrates. Our AP substrate has superior sensitivity, highly rapid and requires no preparation time.

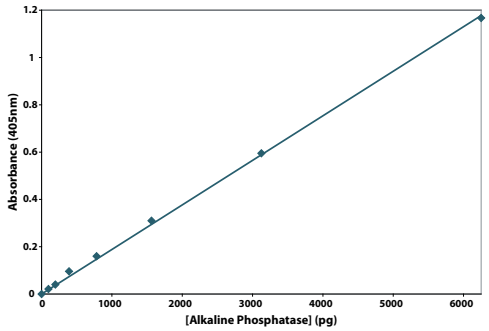


Figure 36: Serial dilutions of alkaline phosphatase incubated with femtoELISA™ for 1 minute.

FEATURES

- Choice of HRP or AP colorimetric substrates
- High sensitivity
- Non-animal blocking agent to minimize cross reactivity

Cat. #	Description	Size
786-110	*femto*ELISA™-HRP Kit	1000 assays
786-111	*femto*ELISA™-HRP substrate only	1000 assays
786-112	*femto*ELISA™-AP Kit	1000 assays
786-113	*femto*ELISA™-AP substrate only	1000 assays

OptiBlaze™ ELISA

High sensitivity chemiluminescence detection

Stabilized ultra sensitive luminol and 1,2 dioxetane based horseradish peroxidase or alkaline phosphatase substrate for the detection of HRP or AP-conjugated antibodies.

The chemiluminescent substrates provided are ultra sensitive substrates developed for luminometer-based applications, specific for horseradish peroxidase or alkaline phosphatase labeled antibodies.

FEATURES

- Stabilized substrates for increased stability
- Detect low femtogram to picogram levels of enzyme
- Premixed solutions

Cat. #	Description	Size
786-302	*OptiBlaze*™ ELISA *femto*-HRP	1000 assays
786-539	*OptiBlaze*™ ELISA *femto*-AP	1000 assays

G-Biosciences Product Line Overview

Protein Research

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

Isolation
- Extraction & Lysis
  - Lysis Buffers
    - Mild Denaturing
    - Strong Chaotropic
    - Specialized
- Fractionation & Enrichment
  - 12 Fractionation Kits
  - Dialysis (Micro)
  - Concentration
- Sample Preparation
  - Contamination Removal
    - Desalting
    - Detergent Removal
    - General Cocktails
    - Species Specific
    - Individual Inhibitors
- Reagents
  - Protease Inhibitors
  - Detergents
  - Chaotropes

Detection
- Electrophoresis
  - 1D & 2D Reagents
    - 2D Specific Kits
    - Buffers & Reagents
  - Gel Stains
    - Coomassie
    - Silver
    - Reversible
- Western Blotting
  - 1 Hour System
  - Blocking Agents
    - Non-Animal
    - Animal
    - Non-Protein
  - Secondary Antibodies
  - Chemiluminescence Detection
- Mass Spectrometry
  - Trypsin, Mass Spec Grade
  - InGel Kits
- Assays (ELISA)
  - Coated Plates
  - Blocking Agents
    - Non-Animal
    - Animal
    - Non-Protein
  - Secondary Antibodies
  - Detection Reagents

Purification
- Affinity Resins
  - 6X His Tag
    - Nickel resin
    - Cobalt resin
    - Copper resin
    - Zinc Resin
    - Glutathione Resin
    - Streptavidin Resin
    - Calmodulin Resin
  - GST Tag
  - Biotin Tag
  - CBP Tag
- Activated Resins
  - Sulfhydryl reactive
  - Amine reactive
  - Carboxyl reactive
  - Drug/ Steroid reactive
- Antibody Purification
  - Protein A or G
  - Pearl Resin
- Labeling
  - Biotin
  - Fluorescent Dye
  - Enzyme (HRP/AP)

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

Antibody
- Production
  - Carrier Proteins
    - BSA
    - KLH
    - HyperCarrier
  - Peptide Coupling
- Purification
  - Protein A or G Resin
  - Activated Resins
  - Pearl Resin
  - Thiophilic Resin
- Fragmentation
  - Ficin
  - Pepsin
  - Papain

BioAssays

- SAM Methyltransferase
- Cell Toxicity & Proliferation
  - Continuous, Enzymatic Assays
  - Lactate Dehydrogenase (LDH)
  - SRB
  - WST-1
- Apoptosis
  - Caspase
    - Assays
    - Substrates
    - Inhibitors
  - Inducers
  - Assays
  - Inhibitors
- Protease
- Phosphatase
- Peroxide
- B-Galactosidase
  - CPRG
  - Fluorescent (MUG)

Molecular Biology

Genomic DNA
- Isolation
  - Tissue
  - Blood
  - Plant
  - Yeast
  - Bacteria
  - Fungi
  - Mouse Tail

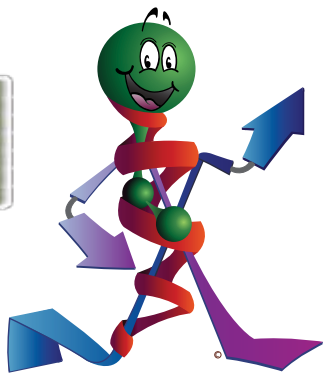
Plasmid DNA
- Isolation
- Colony Screening
- Transformation

Electrophoresis
- Apparatus
- Loading Dyes
- DNA Ladders
- Gel Extraction

PCR
- Taq
- dNTPs

RNA
- Extraction
- RNase Decontamination

Yeast
- Transformation
- Plasmid Isolatio



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