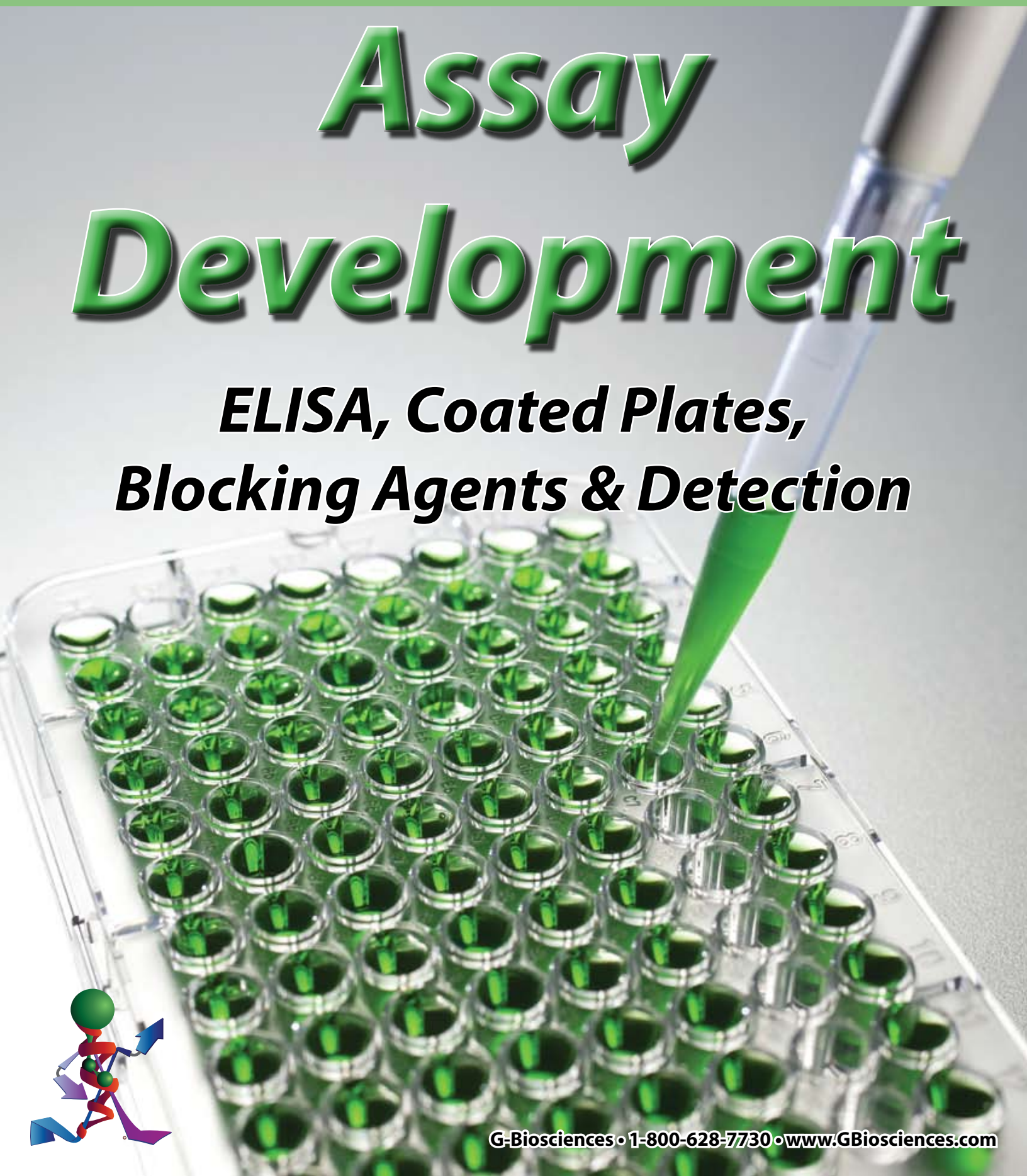
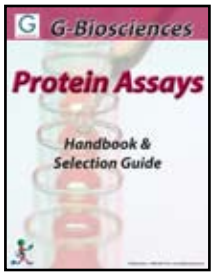
G-Biosciences

Assay Development

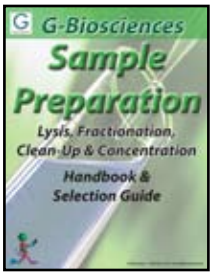
ELISA, Coated Plates, Blocking Agents & Detection

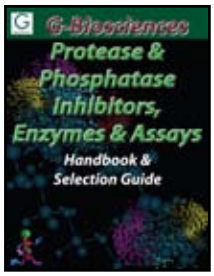




- *Protein Estimation Assays*
- *Apoptosis Assays*
- *Cytotoxicity Assays*
- *SAM Methyltransferase Assays*
- *Protease Assays*
- *Phosphatase Assays*
- *Peroxide Assay*

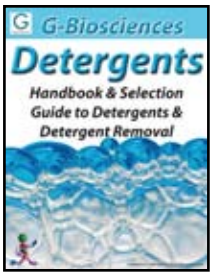
- *Lysis Buffers & Systems*
- *Protein Fractionation Kits*
- *Dialysis (Micro) System*
- *Electrophoresis Clean-Up*
- *Concentration Systems*
- *Contamination Removal*

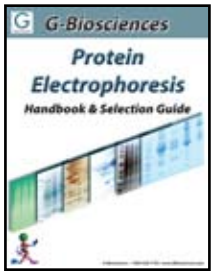




- *Protease Inhibitor Cocktails*
- *Individual Protease Inhibitors*
- *Protease Assays*
- *Proteases for Mass Spec.*
- *Sequencing Grade Proteases*

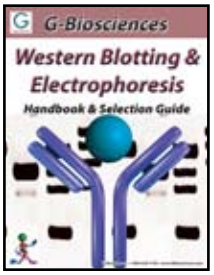
- *Proteomic Grade Detergents*
- *Research Grade Detergents*
- *Non-Ionic, Ionic & Zwitterionic*
- *Detergent Estimations*
- *Detergent Removal Systems*

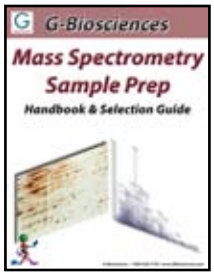




- *Gel Preparation Chemicals*
- *Protein Marker Ladders*
- *Electrophoresis Buffers*
- *Reducing & Alkylating Reagents*
- *Protein Gel Stains*

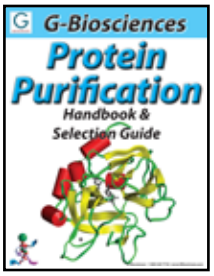
- *1-Hour Western System*
- *Transfer Buffers & Membranes*
- *Membrane Stains*
- *Blocking Buffers*
- *Secondary Antibodies*
- *Detection Reagents*
- *Reprobing Reagents*

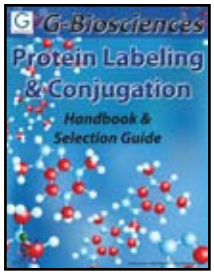




- *Protein Sample Preparation*
- *Protein Clean-Up Systems*
- *Electrophoresis Reagents*
- *Mass Spec Grade Protease*
- *InGel Digestion Kits*
- *Peptide Generation Reagents*

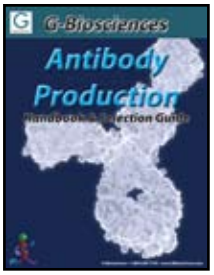
- *Affinity Resins*
- *6X His Protein Purification Kits*
- *GST Protein Purification Kits*
- *Antibody Purification*
- *Activated Resins*
- *Buffers & Reagents*





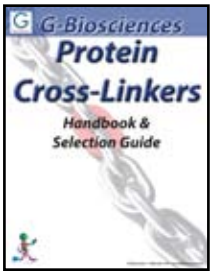
- *Biotin Labeling*
- *Cell Surface Protein Labeling*
- *Agarose Coupling Kits*
- *Fluorescent Dye Labeling Kits*
- *Enzyme Labeling Systems*

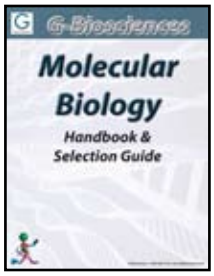
- *Carrier Proteins*
- *Peptide Coupling Systems*
- *Antibody Purification Resins*
- *Antibody Fragmentation Kits*



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

- *Homobifunctional*
- *Heterobifunctional*
- *Optimizer Systems*
- *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*

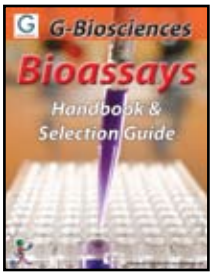


Table of Contents

Introduction

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.

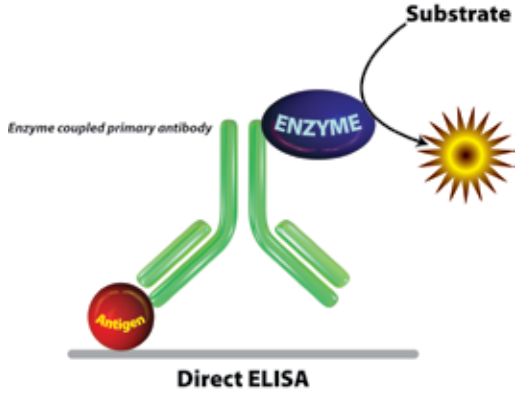


Figure 1: Direct ELISA using a single labeled antibody.

- **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.

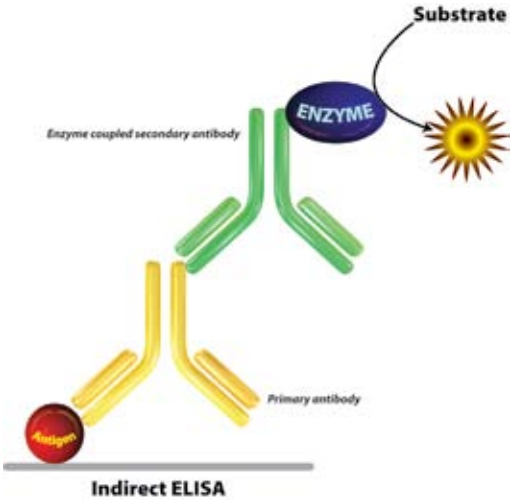


Figure 2: Indirect ELISA using a labeled secondary antibody to detect the primary antibody.

- **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.

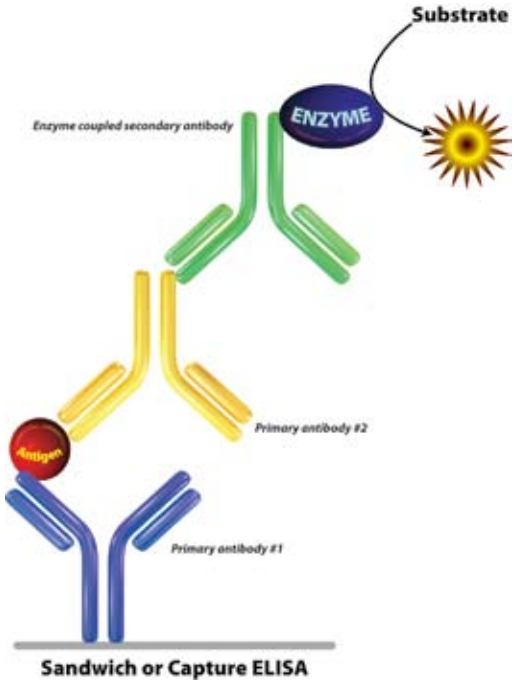


Figure 3: Sandwich ELISA using an antibody to capture the antigen prior to detection.

- **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.

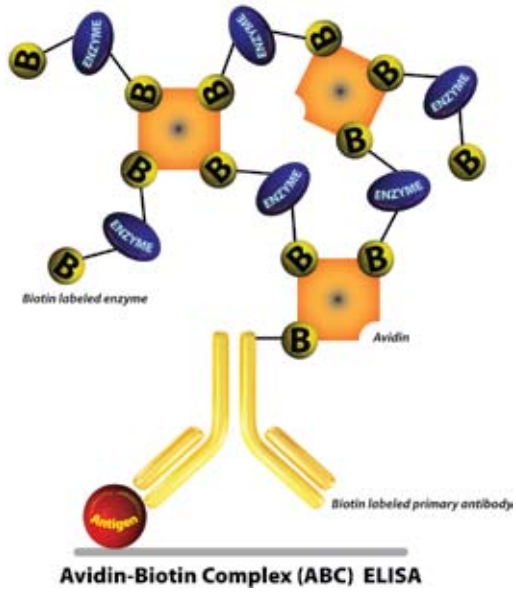


Figure 4: ABC ELISA using an avidn-biotin complex to enhance the ELISA signal.

ELISA PRINCIPLE

Enzyme-Linked ImmunoSorbent Assay (ELISA) is an antibody-based technique, which is used as a fundamental tool in clinical immunology. It is a powerful method for screening of HIV, SARS, etc. and identifying pathogenic agents. It is based on the principle of antibody-antigen interaction, which allows for easy visualization of results and can be completed without the additional concern of radioactive materials use.

ELISA is considered to be a powerful method for estimating nanogram to picogram quantities of antigen or antibody in a solution; such as serum, urine and culture supernatant. ELISA is used in diagnosis and testing of disease, which detects the presence of either antibody or an antigen in the sample.

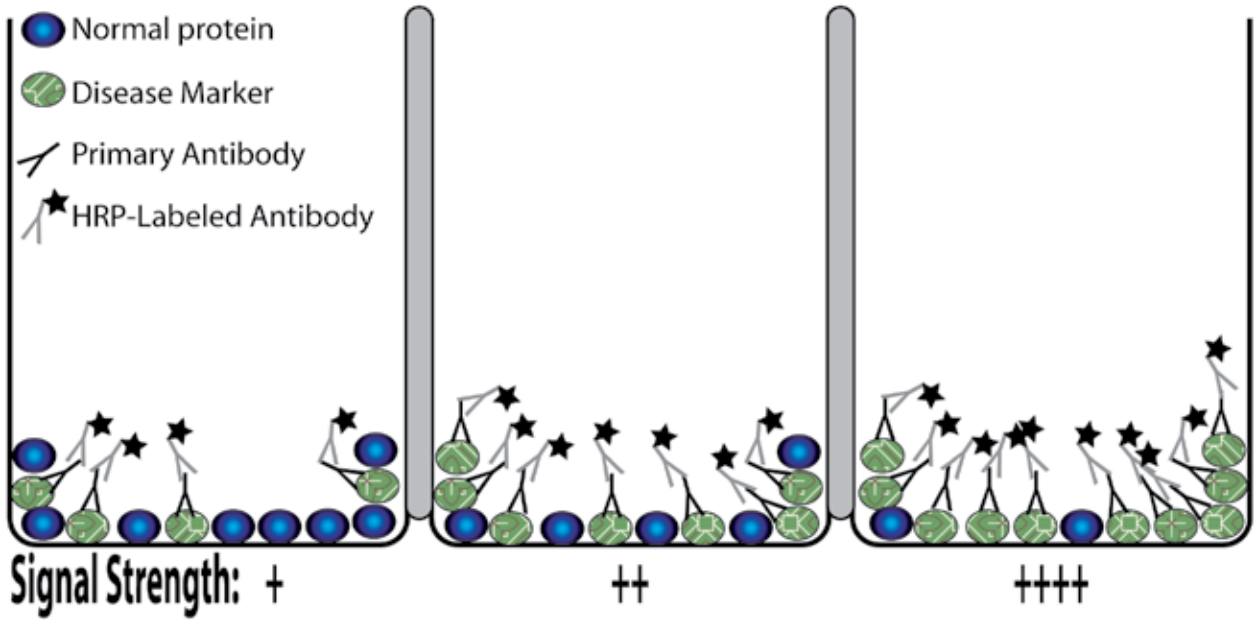
The proteins are immobilized in a protein binding well and non-specific sites are then blocked. The blocking step is used to increase the specificity of the ELISA technique by preventing non-specific interactions. If the wells are not blocked then the antibodies can stick to non-specific proteins due to their charge. To prevent this, the wells are incubated with a protein mixture and the proteins block the charges that would attract the antibodies. Several blocking agents are used, including dried milk powder, bovine serum albumin and casein; however modern blocking agents use synthetic and/or non-animal proteins to prevent any cross reaction with the animal antibodies. An example of a non animal blocker is the provided NAP-Blocker™.

Once blocked, the wells can be probed with a primary antibody, an antibody specific for the protein of interest. Once bound the antibody is visualized, either with a specific tag coupled to the primary antibody or with a secondary antibody. The secondary antibody is a general antibody that recognizes the constant domain of immunoglobulin G and is species specific. So, if the primary antibody is a mouse antibody, the secondary antibody used will recognize all mouse antibodies. If a secondary antibody is used then this will carry the tag that allows visualization of the protein.

The most common tags used are enzymes that catalyze a substrate to produce light or color that is readily detected by plate readers. The enzymes of choice are horseradish peroxidase (HRP) and alkaline phosphatase (AP). The more primary and therefore secondary antibody bound, the greater intensity of the signal. If used in conjunction with standards the ELISA technique is a highly accurate quantitative technique.

ELISA GENERAL PROTOCOL

1. Select an appropriate coated plate *(pages 5-7)* or empty plate.
2. Apply 50-100µl 2-10µg/ml antigen in PBS or TBS *(page 13)*.
3. Incubate, with shaking, at 4°C for 16-20 hours or 37°C for 30-60 minutes.
4. Block unoccupied sites with 200-300µl blocking agent *(page 8)*.
5. Add 100-200µl primary antibody in blocking agent and incubate for >30 minutes.
6. Wash the wells with wash buffers supplemented with Tween® 20 *(page 13)*.
7. Add 100-200µl enzyme conjugated secondary antibody *(page 14)* in blocking agent.
8. Wash the wells with wash buffers supplemented with Tween® 20 *(page 13)*.
9. Add the detection substrate and read the wells with a suitable plate reader *(page 15)*.



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 1: 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).

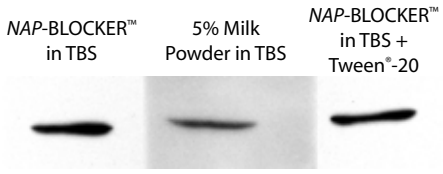


Figure 5: Comparison of NAP-BLOCKER™ and milk powder. Protein lysates were transferred to PVDF membranes and blocked for 90 minutes as indicated. The membranes were probed for actin and subsequently exposed to film for 20 minutes.

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

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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.

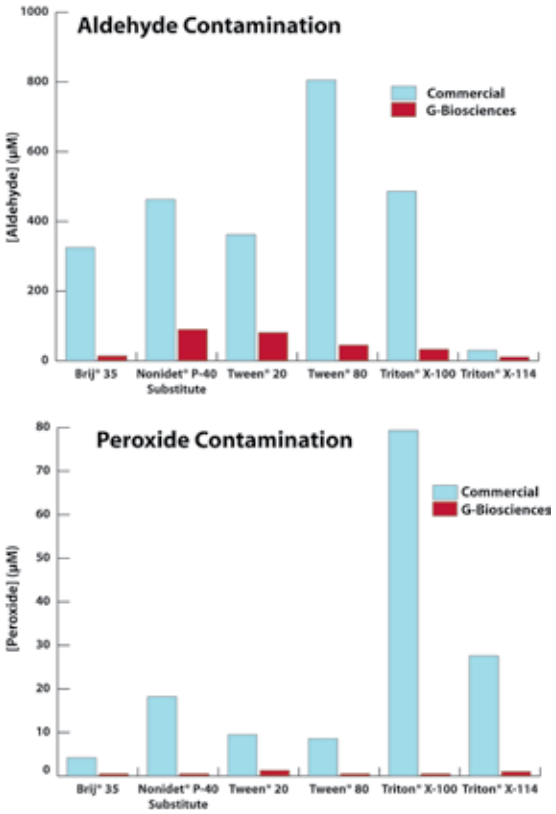


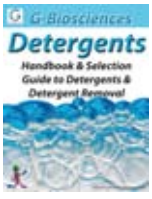
Figure 6: Comparison of aldehyde (top) and peroxide (bottom) concentration in G-Biosciences Proteomic Grade Detergent Solutions and non-proteomic grade commercially available detergents.

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.



Tween® 20

Polyethylene glycol sorbitan monolaurate

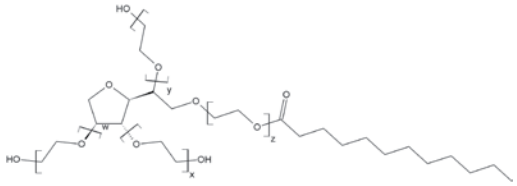


Figure 7: Structure of Tween® 20.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{18}H_{34}O_6 \cdot [C_2H_4O]_{w+x+y+z}$ for w+x+y+z =20
Mol Weight: ~1227 (for w+x+y+z =20)
Absorbance (215nm): 0.05 (0.05% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): approx 0.06 x 10^{-3}M
Cloud Point: 76°C
Application: A commonly used non-ionic detergent for solubilizing membrane proteins during isolation of membrane-protein complexes

Cat. #	Description	Size
DG011	Tween® 20, 10% solution	5 x 10ml vials
DG012	Tween® 20, 10% solution	10 x 10ml vials
DG511	Tween® 20, 10% solution	50ml bottle
DG519	Tween® 20, 10% solution	100ml bottle

Tween® 80

Polyethylene glycol sorbitan monooleate

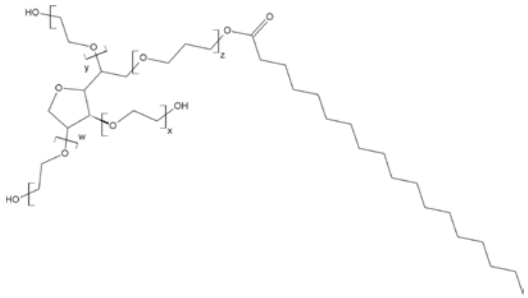


Figure 8: Structure of Tween® 80.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{24}H_{46}O_6 \cdot [C_2H_4O]_{w+x+y+z}$ for w+x+y+z =20
Mol Weight: ~1325 (for w+x+y+z =20)
Absorbance (250nm): 0.14 (0.05% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): ~0.012 x 10^{-3}M (25°C)
Aggregation number: 60
Cloud Point: 65°C
Average micellar weight: 79,000
Application: For solubilizing membrane proteins during isolation of membrane-protein complexes

Cat. #	Description	Size
DG013	Tween® 80, 10% solution	5 x 10ml vials
DG014	Tween® 80, 10% solution	10 x 10ml vials
DG513	Tween® 80, 10% solution	50ml bottle
DG520	Tween® 80, 10% solution	100ml bottle

Triton® X-100

Octylphenolpoly(ethyleneglycolether)x

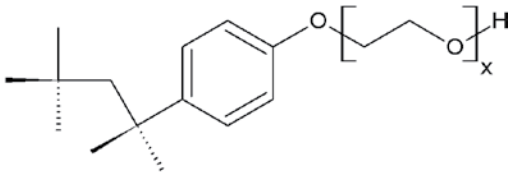


Figure 9: Structure of Triton® X-100.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{34}H_{62}O_{11}$ for x =10
Mol Weight: 647 (for x=10)
Absorbance (254nm): 0.16 (0.05% w/v)
Aldehyde content: <100µM
Peroxide content (as H_2O_2): <50µM
Critical micelle concentration (CMC): approx 0.2 x 10^{-3}M (25°C)
Aggregation number: 100-155
Cloud Point: 65°C
Average micellar weight: 80,000
Application: One of the most commonly used non-ionic detergents for solubilizing membrane proteins during isolation of membrane-protein complexes. Ultra low aldehyde and peroxide concentrations reduce the effects of peroxidase and carbonyl compounds that negatively interact with membrane proteins

Cat. #	Description	Size
DG007	Triton® X-100, 10% solution	5 x 10ml vials
DG008	Triton® X-100, 10% solution	10 x 10ml vials
DG507	Triton® X-100, 10% solution	50ml bottle
DG517	Triton® X-100, 10% solution	100ml bottle

Triton® X-114

Polyethylene glycol tert-octylphenyl ether

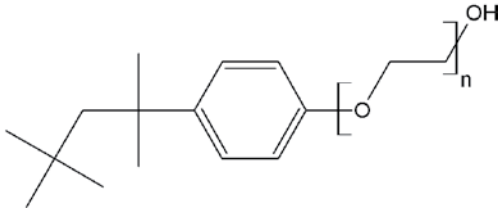


Figure 10: Structure of Triton® X-114.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{14}H_{22}O\bullet[C_2H_4O]_{7-8}$ for n =8
Mol Weight: ~537 (for n=7-8)
Absorbance (254nm): 0.18 (0.05% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): approx 0.35 x 10^{-3}M (25°C)
Cloud Point: 23°C
Application: A non-ionic detergent with a low cloud point (23°C) making it suitable for protein solubilization with phase-partitioning of hydrophilic proteins from amphiphilic proteins

Cat. #	Description	Size
DG009	Triton® X-114, 10% solution	5 x 10ml vials
DG010	Triton® X-114, 10% solution	10 x 10ml vials
DG509	Triton® X-114, 10% solution	50ml bottle
DG518	Triton® X-114, 10% solution	100ml bottle

Brij® 35

Polyoxyethylene (23) lauryl ether

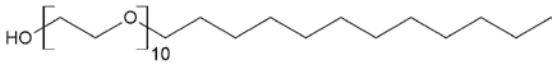


Figure 11: Structure of Brij® 35.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{12}H_{26}O(OCH_2CH_2)_{10}$
Mol Weight: 627
Absorbance (225nm): 0.07 (1% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): 90µM
Aggregation number: 24-40
Cloud Point: >100°C
Average micellar weight: 48,000
Appearance: Clear solution with a faint yellow color
Application: For protein extraction, permeabilization of cells, and preparation of yeast spheroplasts

Cat. #	Description	Size
DG003	Brij® 35, 10% solution	5 x 10ml vials
DG004	Brij® 35, 10% solution	10 x 10ml vials
DG503	Brij® 35, 10% solution	50ml bottle
DG515	Brij® 35, 10% solution	100ml bottle

Brij® 58

Polyoxyethylene (20) cetyl ether

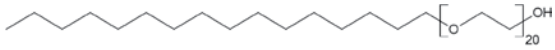


Figure 12: Structure of Brij® 58.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{16}H_{33}(OCH_2CH_2)_{20}$-OH
Mol Weight: 1122
Absorbance (225nm): 0.0788 (1% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): 7-77µM
Aggregation number: 70
Cloud Point: >100°C
Average micellar weight: 79,000
Appearance: Clear solution with a faint yellow color
Application: For protein extraction, permeabilization of cells, and preparation of yeast spheroplasts

Cat. #	Description	Size
DG005	Brij® 58, 10% solution	5 x 10ml vials
DG006	Brij® 58, 10% solution	10 x 10ml vials
DG505	Brij® 58, 10% solution	50ml bottle
DG516	Brij® 58, 10% solution	100ml bottle

Nonidet® P-40 Substitute

Nonylphenyl-polyethylene glycol

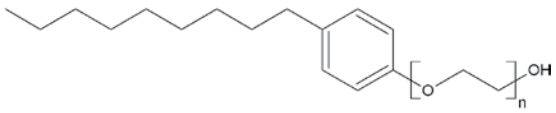


Figure 13: Structure of Nonidet® P-40 Substitute.

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{15}H_{24}O[C_2H_4O]_n$
Mol Weight: 573 (for n=8)
Absorbance (254nm): 0.14 (0.05% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): approx 0.05-0.3mM (25°C)
Application: A commonly used non-ionic detergent for solubilizing membrane proteins during isolation of membrane-protein complexes

Cat. #	Description	Size
DG001	Nonidet® P-40 Substitute, 10% solution	5 x 10ml vials
DG002	Nonidet® P-40 Substitute, 10% solution	10 x 10ml vials
DG501	Nonidet® P-40 Substitute, 10% solution	50ml bottle
DG514	Nonidet® P-40 Substitute, 10% solution	100ml bottle

Proteomic Grade Detergent Variety Pack

The variety pack contains a selection of our non-ionic Proteomic Grade Detergent Solutions, Zwitterionic and non-detergent sulfobetaines for trial and optimization.

The following proteomic grade detergents are available as a trial pack. The pack contains one 10ml vial of 10% aqueous solutions of:

- Triton® X-100
- Triton® X-114
- Tween® 20
- Tween® 80
- Nonidet® P-40 Substitute
- Brij® 35
- Brij® 58

And 1gm of:

- CHAPS
- NDSB 201

Cat. #	Description	Size
DG521	Proteomic Grade Detergent Variety Pack	9 vials

ZWITTERIONIC DETERGENTS

Zwitterionic detergents protect the native state of proteins without altering the native charge of the protein molecules. Zwitterionic detergents are used for isoelectric focusing and 2D electrophoresis. Synthetic zwitterionic detergents are known as sulfobetaines. Sulfobetaines retain their zwitterionic characteristics over a wide range of pH. The following zwitterionic detergents are the most efficient and widely used for 2D gel electrophoresis.

CHAPS

3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate

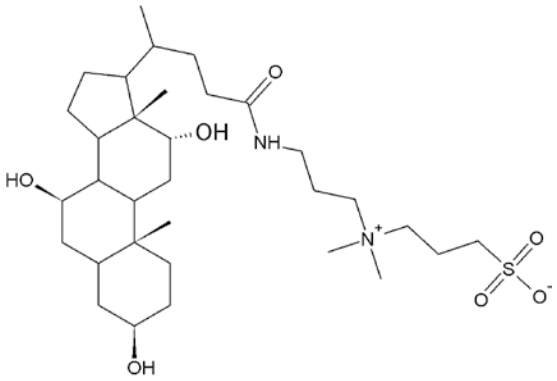


Figure 14: Structure of CHAPS.

Type: Zwitterionic detergent
Mol. Formula: $C_{32}H_{58}N_2O_7S$
Mol Weight: 614.9
Form: White solid
Purity: >99%
Solubility: Water soluble
Conductivity: <25µS in a 10% solution
Critical micelle concentration (CMC): 6-10mM (25°C)
Aggregation number: 10
Cloud point: >100°C
Average micellar weight: 6150
Application: Zwitterionic detergent. Non-denaturing. Electrically neutral. CHAPS has all the advantages of sulfobetaine containing detergents: hydrophobic, bile salt, and anionic detergents in a single molecule. Better at solubilizing proteins and breaking protein-protein interactions. Less protein aggregation than non-ionic detergents. Capable of solubilizing opiate receptors. CHAPS can be removed from protein solutions with a detergent removing gel or by dialysis

Cat. #	Description	Size
DG049	CHAPS	1g
DG050	CHAPS	5g
DG051	CHAPS	25g
DG096	CHAPS	100g

CHAPSO

3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate

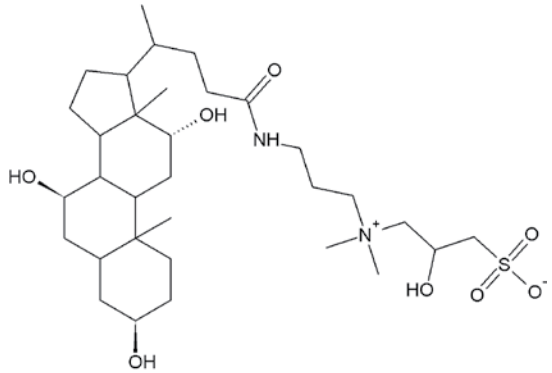


Figure 15: Structure of CHAPSO.

Type: Zwitterionic detergent
Mol. Formula: $C_{32}H_{58}N_2O_8S$
Mol Weight: 630.9
Form: White solid
Purity: >99%
Solubility: Water soluble
Conductivity: <50µS in a 10% solution
Critical micelle concentration (CMC): 8mM (25°C)
Aggregation number: 11
Cloud point: 90°C
Average micellar weight: 7000
Application: Zwitterionic detergent. Non-denaturing. Electrically neutral. Higher solubility than CHAPS because of a more polar head group. Solubilizes membrane proteins in their native state. Solubilizes opiate receptor to a state exhibiting reversible binding of opiates

Cat. #	Description	Size
DG052	CHAPSO	1g
DG053	CHAPSO	5g

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

ENZYME LABELING

For the rapid and stable coupling of HRP and AP enzymes to proteins

The HOOK™ Enzyme Labeling Kits are designed for the coupling of horseradish peroxidase (HRP) and alkaline phosphatase (AP) to proteins, particularly antibodies.

G-Biosciences offers three enzyme labeling kits that are supplied with all the reagents required for high efficiency enzyme coupling.

HOOK™ HRP PLUS Labeling

A high efficiency enzyme labeling kit for tagging proteins with horseradish peroxidase enzyme. This kit has an activated HRP that couples with high efficiency (>90%) to the numerous amine groups of proteins and is superior to glutaraldehyde coupling chemistry.

Uses HOOK™ HRP PLUS, which is HRP that has been activated by the addition of reactive aldehydes. The aldehyde groups react spontaneously and at high efficiency with primary amines, located at the N-terminus of proteins or in lysine residues, to form intermediate Schiff Base complexes. These, in turn, are selectively reduced by the supplied reduction agent. Following quenching of the reaction the protein is linked to the horseradish peroxidase enzyme by stable amine linkage. The labeled protein, or antibody, can now be used for immunoblotting, ELISA and histochemical techniques.

FEATURES

- Activity is 120-200 units/mg
- Reacts with primary amines to form covalent amine bonds

Cat. #	Description	Size
786-313	HOOK™ HRP PLUS labeling kit	5 reactions

HOOK™ HRP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with horseradish peroxidase (HRP) enzyme. This kit has activated HRP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated HRP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of HRP conjugates using free sulfhydryls the kit is supplied with SATA (N-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating HRP to immunoglobulin G (IgG) and its fragments.

Cat. #	Description	Size
786-314	HOOK™ HRP SULFO labeling kit	5 reactions

HOOK™ AP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with alkaline phosphatase enzyme. This kit has activated AP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated AP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of AP conjugates using free sulfhydryls the kit is supplied with SATA (N-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating AP to immunoglobulin G (IgG) and its fragments.

Cat. #	Description	Size
786-315	HOOK™ AP SULFO labeling kit	5 reactions

FLUORESCENT DYE LABELING

The labeling of proteins with fluorescent dyes has become an important research tool in many fields. Two kits are offered for labeling virtually any protein, particularly antibodies, with either a rhodamine or fluorescein based dye.

HOOK™ Dye Labeling Kit (5/6) TAMRA-SE (Rhodamine)

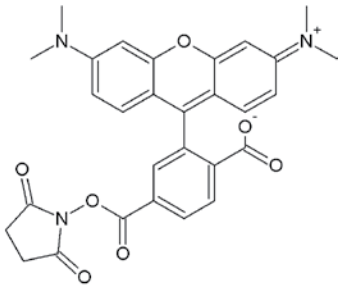


Figure 16: Structure of (5/6) TAMRA-SE.

(5/6) TAMRA-SE (5-(and-6)- Carboxytetramethylrhodamine succinimidyl ester, mixed isomers) is based on tetramethylrhodamine, one of the most common fluorophores used in the labeling of peptides, proteins, nucleic acids and nucleotides.

(5/6) TAMRA absorbs green visible light at 546nm and emits an orange-red visible light at a maximum emission of 575nm.

The NHS ester group provides the simplest and most commonly used group for labeling proteins. The succinimidyl ester group reacts with primary amines in lysine side chains and N-terminal amines forming a stable, covalent amide bond.

This kit utilizes SpinOUT™ columns for the rapid purification of dye labeled proteins.

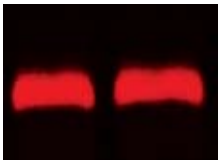


Figure 17: Visualization of TAMRA labeled BSA. 1µg (5/6) TAMRA-SE labeled BSA was resolved on a 4-20% SDS polyacrylamide gel.

FEATURES

- Dye preweighed and supplied in single use OneQuant™ vials
- Suitable for most proteins
- Utilizes SpinOUT™ desalting columns to isolate labeled protein

APPLICATIONS

- Labeling of proteins, peptides and nucleic acids with a red fluorescent dye
- Suitable for antibody labeling

Cat. #	Description	Size
786-142	HOOK™ (5/6) TAMRA-SE (Rhodamine) Labeling Kit	1 kit

HOOK™ Dye Labeling Kit (FITC)

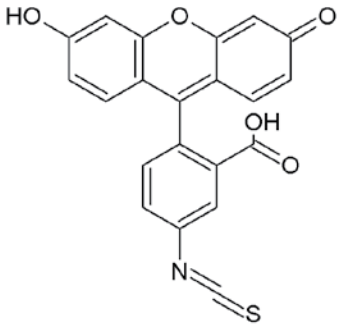


Figure 18: Structure of fluorescein isothiocyanate.

FITC (fluorescein isothiocyanate) is a commonly used fluorescent label for proteins, as it contains the groups required for conjugating to amino, sulfhydryl, imidazoyl, tyrosyl or carbonyl groups of proteins. FITC has a molecular weight of 389, and excitation and emission wavelengths of 494nm and 520nm, respectively, therefore emitting green visible light.

This kit utilizes SpinOUT™ columns for the rapid purification of dye labeled proteins.

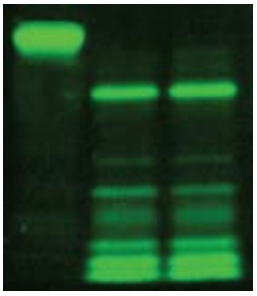


Figure 19: Visualization of FITC Labeled Casein. Lane 1: 1µg FITC labeled casein, Lane 2-3: 1µg FITC-Casein digested with 0.2µg or 0.1µg Trypsin. Samples were resolved on a 4-20% SDS polyacrylamide gel.

FEATURES

- Dye preweighed and supplied in single use OneQuant™ vials
- Suitable for most proteins
- Utilizes SpinOUT™ desalting columns to isolate labeled protein

APPLICATIONS

- Labeling of a green fluorescent dye to proteins and peptides
- Suitable for antibody labeling

Cat. #	Description	Size
786-141	HOOK™ FITC Labeling Kit	1 kit

OneQuant™ Fluorescent Reagents

Both the fluorescent reagents (FITC and (5/6) TAMRA) are available in our *OneQuant*™ format.

The *OneQuant*™ format prevents loss of reagent due to repeated weighing. Each vial also limits exposure to light.

Cat. #	Description	Size
786-079	OneQuant™ TAMRA	8 x 0.5mg
786-080	OneQuant™ FITC	8 x 1mg

BIOTIN LABELING

Ideal for ELISA detection and ABC ELISAs

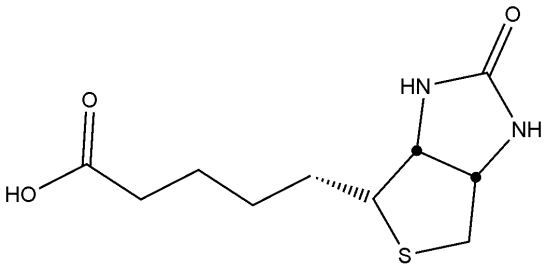


Figure 20: Structure of Biotin.

Biotin, a 244 Dalton vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ($K_a=10^{15}M^{-1}$) and streptavidin. 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. The biotinylated molecules are efficiently probed with avidin or streptavidin conjugated to reporter molecules, such as peroxidases or phosphatases.

Several factors must be considered when coupling a biotin reagent to a protein to ensure a successful reaction. The primary consideration is the selection of the biotinylation reagent itself. A wide range of biotin reagents are offered that have variations in their reactive groups, spacer arm lengths, solubility, membrane permeability and reversibility. All these factors must be considered and are dependent on your protein/peptide.

- **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.

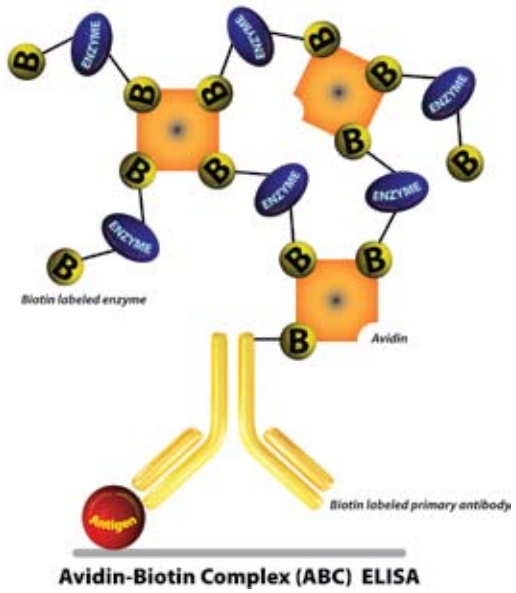


Figure 21: ABC ELISA using an avidn-biotin complex to enhance the ELISA signal.

HOOK™ IgG Biotinylation

Rapid antibody labeling with biotin

Designed for the efficient biotinylation of IgG molecules by first immobilizing the IgG molecules on a solid support

The HOOK™ IgG Biotinylation kits offer an advantage over standard biotinylation reactions as the immobilization of the IgG to the Nickel Chelating resin allows for the rapid removal of uncoupled biotin and therefore eliminates the need for further dialysis or desalting of the biotinylated antibody.

Two kits are available for labeling antibodies through free amines or sulfhydryls. The amine kit uses NHS-dPEG$_4$-Biotin to label free primary amines. The sulfhydryl kit uses the supplied Protein-S-S-Reductant™ to reduce the disulfide bonds of the immobilized IgG molecule. The reduced immobilized IgG molecule is then incubated with PEG$_2$-Iodoacetyl-Biotin solution to biotinylate the free sulfhydryl groups.

The advantage of a PEG (polyethylene glycol) biotinylation reagent is that the long hydrophilic spacer arm conveys its water solubility to the antibodies and have a reduced occurrence of aggregation compared to non-PEG biotinylation reactions.

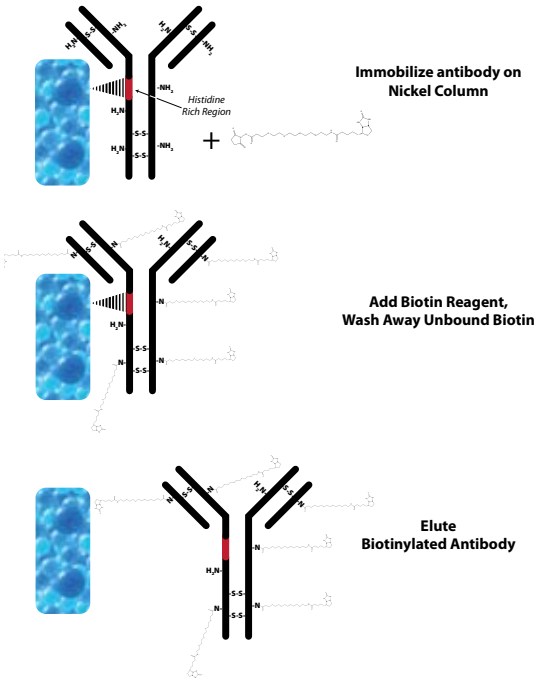


Figure 22: HOOK™ IgG Biotinylation (Amine) Scheme. The IgG antibody is first immobilized through its histidine rich domain on a nickle column. Immobilized antibody is labeled with the NHS-dPEG$_4$-Biotin reagent that reacts with primary amines. Free biotin is washed away and the biotinylated antibody is eluted with the supplied His Elution Buffer.

FEATURES

- Simpler antibody biotinylation
- Solid support technology eliminates dialysis/desalting
- Suitable for 1-10mg antibody
- PEG Biotin reagent for reduced steric hindrance and increased labeled antibody solubility

APPLICATIONS

- For the efficient and simple labeling of antibodies with biotin

Cat #	Description	Size
786-728	HOOK™ IgG Biotinylation (Amine)	8 reactions
786-729	HOOK™ IgG Biotinylation (Sulfhydryl)	8 reactions

HOOK™ Biotin Kits

For highly efficient labeling of proteins

HOOK™ Biotin kits come with all the necessary reagents, equipment and instructions for optimization of reaction conditions, efficient labeling, removal of unbound biotin and quantification of biotin labeling. In addition to highly efficient labeling, the HOOK™ Biotin kits offer the advantage of being supplied with SpinOUT™ desalting columns and a specific Optimizer Buffer™. These simplify the labeling process and ensure high levels of biotin labeling.

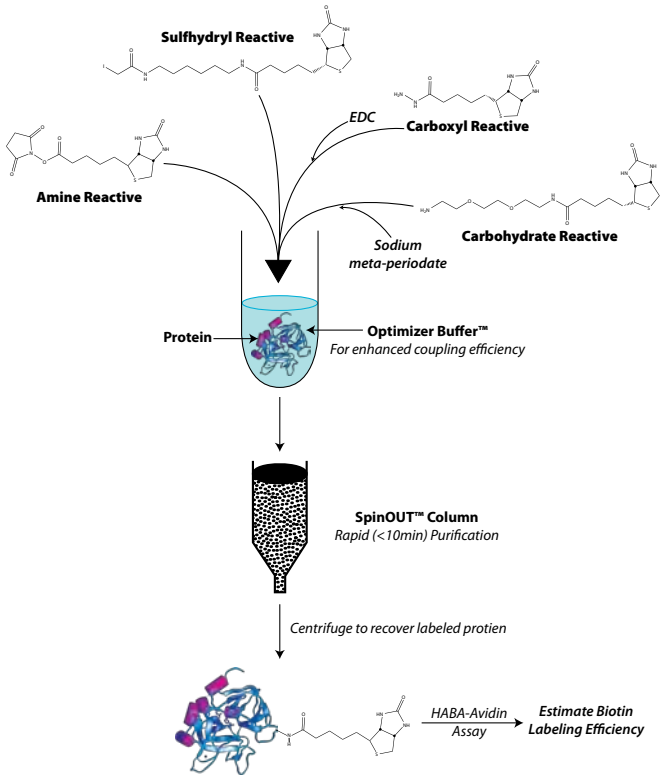


Figure 23: HOOK™ Biotin kit scheme.

PROTEIN LABELING

Each kit is supplied with 25mg of specific HOOK™ Biotin Reagent that conjugates to proteins through amines, sulfhydryls, carboxyls or carbohydrates. The amine and sulfhydryl coupling HOOK™ Biotin Reagents couple directly to the protein through their reactive groups, however the carboxyl coupling HOOK™ Biotin Reagents require a carbodiimide crosslinker and the carbohydrate coupling HOOK™ Biotin Reagents require carbohydrate oxidation before coupling. The HOOK™ Biotin kits include EDC as the carbodiimide crosslinker in the carboxyl coupling kits and sodium meta-periodate for carbohydrate oxidation in the carbohydrate coupling kits.

In addition to the above, each HOOK™ Biotin kit contains a specific Optimizer Buffer that provides the optimal reaction conditions for each HOOK™ Biotin Reagent.

PURIFICATION

Following the labeling of the protein with the HOOK™ Biotin Reagent the unreacted biotin and other chemicals are rapidly removed from the labeled protein with the supplied SpinOUT™ columns. These columns use gel filtration to remove the by-products in <10 minutes.

BIOTIN ESTIMATION

HOOK™ BiotinQuant measures biotin using HABA [4′-hydroxyazobenzene-2-carboxylic acid] dye. HABA binds with avidin at the biotin-binding site. A characteristic color, that absorbs at 500nm, is produced (ε=35,500 $M^{-1}cm^{-1}$ expressed as per mole of HABA bound). Biotin or biotinylated agents compete with the HABA for the binding sites and the greater affinity biotin reagents displace HABA from the avidin binding sites and proportionally reduce the absorbance. The HOOK™ BiotinQuant kit is supplied with each HOOK™ Biotin Kit and is also available separately. The HABA dye is also available separately.

FEATURES

- Optimizer Buffer™ for improved coupling efficiency
- SpinOUT™ gel filtration columns for rapid (<10 minute) purification
- Biotin assay reagents to determine level of biotin incorporation
- Labels 1-10mg protein/reaction
- Suitable for 10 coupling reactions

Cat. #	Description	Size
BS-01	HOOK™-NHS-Biotin Kit	10 reactions
BS-02	HOOK™-NHS-LC-Biotin Kit	10 reactions
BS-03	HOOK™-NHS-LC-LC-Biotin Kit	10 reactions
BS-04	HOOK™-NHS-SS-Biotin Kit	10 reactions
BS-05	HOOK™-NHS-$dPEG_4$™-Biotin Kit	10 reactions
BS-06	HOOK™-sulfo-NHS-Biotin Kit	10 reactions
BS-07	HOOK™-sulfo-NHS-LC-Biotin Kit	10 reactions
BS-08	HOOK™-sulfo-NHS-LC-LC-Biotin Kit	10 reactions
BS-09	HOOK™-sulfo-NHS-SS-Biotin Kit	10 reactions
BS-10	HOOK™-PFP-Biotin Kit	10 reactions
BS-11	HOOK™-PEG_2-Iodoacetyl-Biotin Kit	10 reactions
BS-12	HOOK™-Iodoacetyl-LC-Biotin Kit	10 reactions
BS-13	HOOK™-Biotin-PDA Kit	10 reactions
BS-14	HOOK™-Biotin-BMCC Kit	10 reactions
BS-15	HOOK™-Biotin-Pentylamine Kit	10 reactions
BS-16	HOOK™-Biotin-PEG_2-Amine Kit	10 reactions
BS-17	HOOK™-Biotin-PEG_3-LC-Amine Kit	10 reactions
BS-18	HOOK™-Biotin-Hydrazide Kit	10 reactions
BS-19	HOOK™-Biotin-LC-Hydrazide Kit	10 reactions

HOOK™ BiotinQuant

For the estimation of biotin conjugation

HOOK™ BiotinQuant measures biotin using HABA [4′-hydroxyazobenzene-2-carboxylic acid] dye. HABA binds with avidin at the biotin-binding site. A characteristic color, that absorbs at 500nm, is produced (ε=35,500 M^{-1} cm^{-1} expressed as per mole of HABA bound). Biotin or biotinylated agents compete with the HABA for the binding sites and the greater affinity biotin reagents displace HABA from the avidin binding sites and proportionally reduce the absorbance.

Cat. #	Description	Size
BKC-01	HOOK™ BiotinQuant Kit	20 assays
BKC-03	HABA Dye	1g

Micro HOOK™ Biotin Kits

For highly efficient labeling of proteins

The micro HOOK™ Biotin kits are designed to label small amounts of proteins, with each kit designed for 8-10 labelings of 50-250µg protein/reaction. Each kit is supplied with all the necessary reagents for optimization of reaction conditions, efficient labeling and removal of unbound biotin. In addition to highly efficient labeling, the HOOK™ Biotin kits offer the advantage of being supplied with SpinOUT™ desalting columns and a specific Optimizer Buffer™. These simplify the labeling process and ensure high levels of biotin labeling.

PROTEIN LABELING

Each kit is supplied with 8 x 1mg single use aliquots of biotin reagent to minimize waste and degradation of the NHS ester coupling reaction group. The following HOOK™ Biotin reagents are available in the micro format:

- **HOOK™ Sulfo-NHS-Biotin**
  Amine reactive reagent, shortest spacer arm
- **HOOK™ Sulfo-NHS-LC-Biotin**
  Amine reactive reagent, longer spacer arm
- **HOOK™ Sulfo-NHS-SS-Biotin**
  Cleavable, amine reactive reagent
- **HOOK™ NHS-$dPEG_4$-Biotin**
  Amine reactive, pegylated reagent; enhances water solubility

In addition, each HOOK™ Biotin kit contains a specific Optimizer Buffer™ that provides the optimal reaction conditions.

PURIFICATION

Following the labeling of the protein with the HOOK™ Biotin Reagent the unreacted biotin and other chemicals are rapidly removed from the labeled protein with the supplied SpinOUT™ Columns. These columns use gel filtration to remove the by-products in <10 minutes.

FEATURES

- Micro kit for labeling protein primary amines
- Optimizer Buffer™ for improved coupling efficiency
- Gel filtration columns for rapid (<10 minute) purification
- Labels 50-250µg protein/reaction
- Suitable for 8-10 couplings

Cat. #	Description	Size
786-694	HOOK™-sulfo-NHS-Biotin Kit (micro)	8-10 reactions
786-695	HOOK™-sulfo-NHS-LC-Biotin Kit (micro)	8-10 reactions
786-696	HOOK™ Sulfo-NHS-SS-Biotin Kit (micro)	8-10 reactions
786-697	HOOK™-NHS-$dPEG_4$-Biotin Kit (micro)	8-10 reactions

HOOK™ Biotin Reagents

To select a biotin reagent several factors need to be considered:

- **Reactive Group:** Determines the location of the biotin moiety
- **Membrane Permeability:** For cell surface labeling select non membrane permeable reagents
- **Cleavable:** For easy removal from immobilized avidin or streptavidin during purification
- **Reversible:** An alternative to cleavable reagents are reversible reagents
- **Steric Hinderance:** Bulky groups around the binding site may require reagents with longer spacer arms.

Cat. #	HOOK™ Biotin Reagent	Size	Molecular Weight	Spacer Arm (Å)	Reactive Group	Membrane Permeable	Water Soluble	Cleavable/ Reversible	Reaction pH
BG-00	**d-Biotin (vitamin H)**	500mg	244.32	0					
AMINE REACTIVE REAGENTS									
BG-01 786-083	**HOOK™-NHS-Biotin**	50mg 8 x 2mg	341.38	13.5	NHS-ester	YES	NO	NO	7-9
BG-02	**HOOK™-NHS-LC-Biotin**	50mg	454.54	22.4	NHS-ester	YES	NO	NO	7-9
BG-03	**HOOK™-NHS-LC-LC-Biotin**	50mg	567.70	30.5	NHS-ester	YES	NO	NO	7-9
BG-04	**HOOK™-NHS-SS-Biotin**	50mg	504.65	24.3	NHS-ester	YES	NO	YES	7-9
BG-05 786-700	**HOOK™ NHS-dPEG$_4$ -Biotin**	50mg 8 x 1mg	588.67	29	NHS-ester	NO	YES	NO	7-9
BG-06 786-698	**HOOK™-sulfo-NHS-Biotin**	50mg 8 x 1mg	443.43	13.5	sulfo-NHS ester	NO	YES	NO	7-9
BG-07 786-084	**HOOK™-sulfo-NHS-LC-Biotin**	50mg 8 x 1mg	556.59	22.4	sulfo-NHS ester	NO	YES	NO	7-9
BG-08	**HOOK™-sulfo-NHS-LC-LC-Biotin**	50mg	669.75	30.5	sulfo-NHS ester	NO	YES	NO	7-9
BG-09 786-699	**HOOK™-sulfo-NHS-SS-Biotin**	50mg 8 x 1mg	606.69	24.3	sulfo-NHS ester	NO	YES	YES	7-9
BG-10	**HOOK™-PFP-Biotin**	50mg	410.36	9.6	Pentafluorophenyl ester	YES	NO	NO	7-9
SULFHYDRYL REACTIVE REAGENTS									
BG-11	**HOOK™-PEG$_2$-Iodoacetyl-Biotin**	50mg	542.43	24.7	Iodoacetyl	NO	YES	NO	7.5-8.5
BG-12 786-085	**HOOK™-Iodoacetyl-LC-Biotin**	50mg 8 x 2mg	510.43	27.1	Iodoacetyl	YES	NO	NO	7.5-8.5
BG-13	**HOOK™-Biotin-PDA**	50mg	412.60	21.1	Pyridyldithiol	YES	NO	YES	6-9
BG-14	**HOOK™-Biotin-BMCC**	50mg	533.68	32.6	Maleimide	NO	NO	NO	6.5-7.5
CARBOXYL REACTIVE REAGENTS									
BG-15	**HOOK™-Biotin-Pentylamine**	50mg	328.47	18.9	Amine	NO	YES	NO	4-6
BG-16	**HOOK™-Biotin-PEG$_2$-Amine**	50mg	374.50	20.4	Amine	NO	YES	NO	4-6
BG-17	**HOOK™-Biotin-PEG$_3$-Amine**	50mg	418.55	22.9	Amine	NO	YES	NO	4-6
CARBOHYDRATE REACTIVE REAGENTS									
BG-18	**HOOK™-Biotin-Hydrazide**	50mg	258.34	15.7	Hydrazide	YES	NO	NO	4-6
BG-19	**HOOK™-Biotin-LC-Hydrazide**	50mg	371.50	24.7	Hydrazide	YES	NO	NO	4-6
PHOTOREACTIVE REAGENTS									
BG-20	**HOOK™-Psoralen-PEO-Biotin**	5mg	688.79	36.9	Psoralen	NO	YES	NO	4-6

BIOTIN PURIFICATION

Streptavidin & Avidin Resins

High binding affinity for biotin labeled proteins & molecules

Biotin, a 244Da vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ($Ka=10^{15}\ M^{-1}$) and streptavidin ($Ka=10^{15}\ M^{-1}$). Biotin and (strept)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•HCl at pH1.5 or by boiling in SDS Page Sample Loading Buffer.

Avidin is a glycoprotein with approximately 10% of its total mass coming from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each has a single biotin binding domain. Avidin is a basic protein with an isoelectric pH of 10-10.5 and is readily soluble in aqueous buffers containing a wide range of salt, pH (2-11), temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications. Avidin has extraordinary binding affinity for biotin ($Ka=10^{15}M^{-1}$).

Streptavidin is a tetrameric protein containing 4 biotin binding sites. Streptavidin in many respects is similar to avidin except that it has no carbohydrate and has a slightly lower molecular weight of about 60kDa. The solubility of streptavidin (isoelectric pH5) in aqueous buffer is much lower than avidin, but the binding of streptavidin to biotin is similar to that of avidin. The advantage of streptavidin is that the lack of carbohydrates significantly reduces the amount of non-specific binding. The streptavidin used for immobilization on porous 6% crosslinked agarose is a recombinant form with a mass of 53kDa and near neutral pI. The streptavidin in covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The resins are designed for the single step small and large scale affinity purification of proteins and antibodies with a biotin tag. The resins can also be used for immunoprecipitations using biotin labeled antibodies. Specific Binding and Elution Buffers are also available.

FEATURES

- Avidin covalently coupled to ~6% cross linked agarose
- Recombinant streptavidin covalently coupled to ~6% cross linked agarose
- Minimal Leaching
- Ligand Density >1mg/ml
- Binding capacity 15-30µg biotin/ml resin

APPLICATIONS

- Immunoprecipitation with biotinylated antibodies
- Pull down assays with biotinylated proteins
- Purification of biotinylated molecules, including:
  - Proteins
  - Antibodies
  - DNA
  - Carbohydrates

Cat #	Description	Size
786-593	Immobilized Avidin Resin	5ml resin
786-594	Immobilized Avidin Resin	25ml Resin
786-590	Immobilized Streptavidin Resin	2ml resin
786-390	Immobilized Streptavidin Resin	5ml Resin
786-591	Immobilized Streptavidin Resin	10ml resin
786-592	Immobilized Streptavidin Resin	5 x 1ml
786-548	Streptavidin Binding Buffer	100ml
786-549	Streptavidin Elution Buffer	100ml

Monomeric Avidin Resin

Purification & elution of biotin labeled molecules under mild elution conditions

G-Biosciences Immobilized Monomeric Avidin Resin is designed for the simple affinity chromatography purifications of proteins, antibodies and other molecules with a biotin tag. The resin consists of monomeric subunits of avidin covalently coupled to 6% cross-linked agarose, offering a stable, reusable resin for the purification of biotinylated molecules.

Monomeric avidin offers a distinct advantage over native avidin, a tetrameric molecule, and streptavidin as it has a much lower biotin binding affinity, $Kd=10^{-7}$ as opposed to $Kd=10^{-15}$ for native avidin. This lower binding affinity allows elution of molecules with mild elution buffers (2mM D-Biotin in 1X PBS), as opposed to the strong denaturing buffers (8M Guanidine•HCl, pH 1.5) used with native avidin.

The covalent attachment of monomeric avidin to the agarose ensures no detectable leaching of the avidin during biotin purification and offers a wide tolerance to chemicals. This ensures the resin can be reused at least 10 times with no loss of function.

The Immobilized Monomeric Avidin Resin is available as a 50% resin slurry or as a complete kit containing a reusable monomeric avidin column and the respective buffers for successful purification of biotinylated molecules.

FEATURES

- Monomeric avidin covalently coupled to ~6% cross linked agarose.
- Minimal Leaching
- Binding capacity »1.2mg biotinylated BSA/ml resin
- Non Denaturing: Elute biotinylated molecules with free biotin
- Reusable: Reuse the resin at least 10 times (2.5% loss of binding/ regeneration)
- Specific: Retains avidins high specificity for biotin molecules

APPLICATIONS

- Purification of biotinylated molecules, including:
  - Proteins
  - Antibodies
  - DNA
  - Carbohydrates

Cat #	Description	Size
786-595	Immobilized Monomeric Avidin	5ml resin
786-596	Immobilized Monomeric Avidin	10ml resin
786-597	Immobilized Monomeric Avidin	Kit

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.

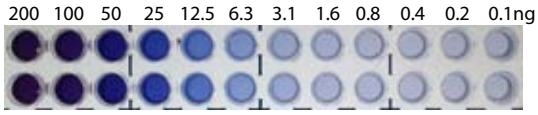


Figure 24: Serial dilutions of HRP incubated with our ELISA substrate for 10 minutes.

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 (Figure 130), 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 (Figure 131) and requires no preparation time.

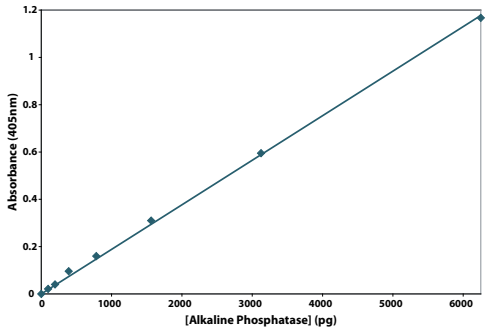


Figure 25: Serial dilutions of alkaline phosphatase incubated with femtoELISA™ for 1 minute.

FEATURES

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

APPLICATIONS

- ELISA kits and substrates for highly sensitive colorimetric detection of HRP or AP.

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

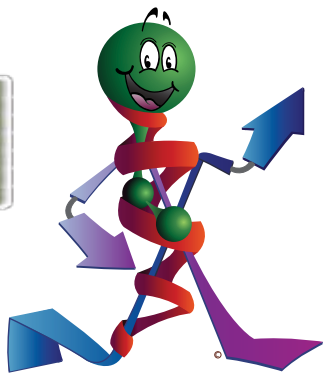
Plasmid DNA
- Isolation
- Colony Screening
- Transformation

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

PCR
- Taq
- dNTPs

RNA
- Extraction
- RNase Decontamination

Yeast
- Transformation
- Plasmid Isolatio



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