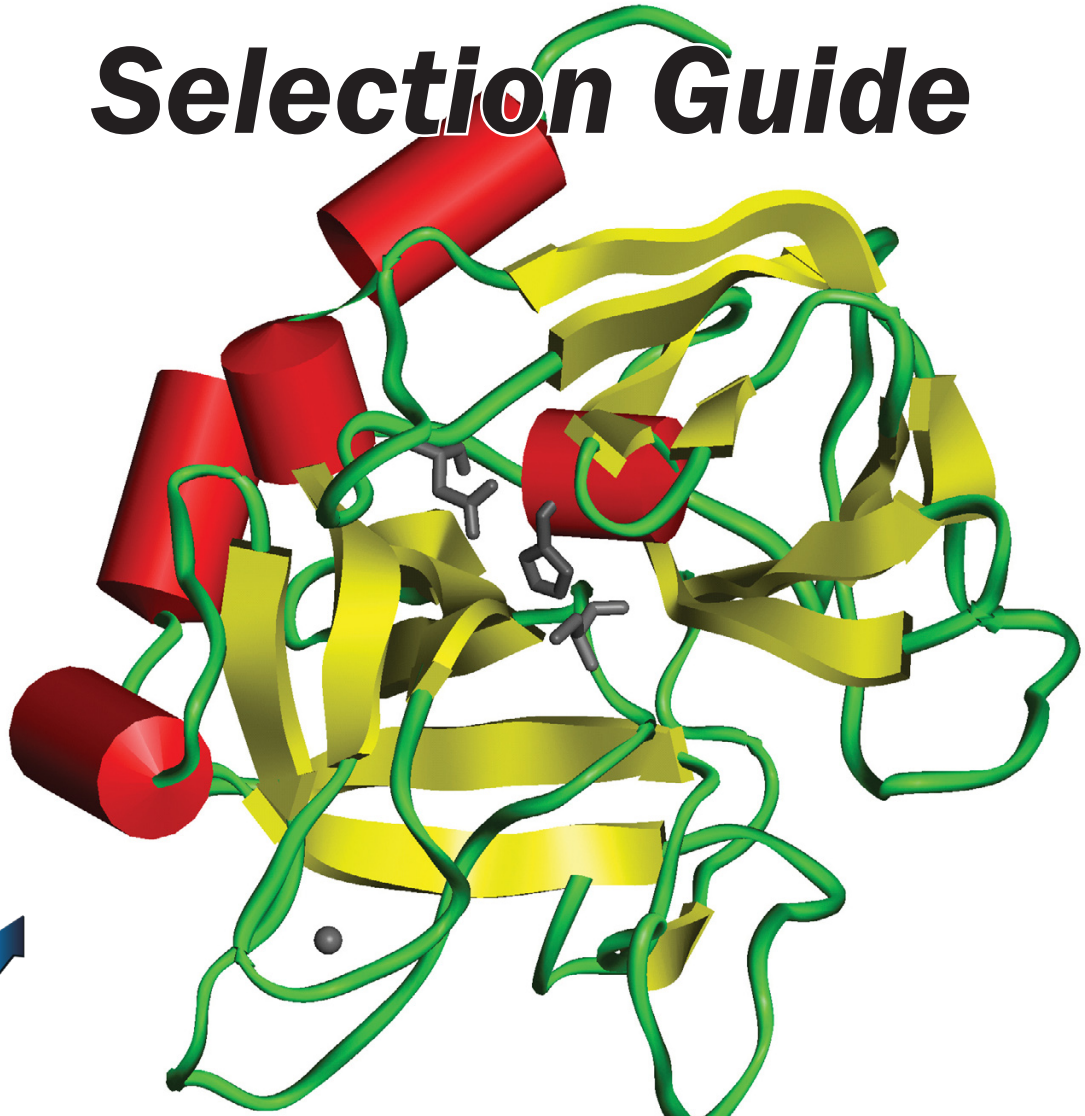
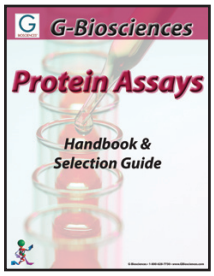
G-Biosciences

Protein Purification

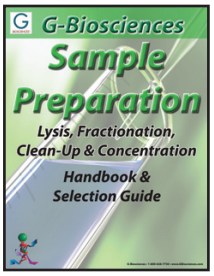
Handbook & Selection Guide

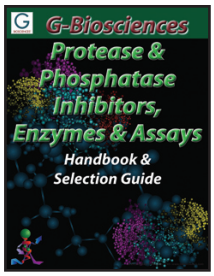




- Protein Estimation Assays
- Apoptosis Assays
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- Protease Assays
- Phosphatase Assays
- Peroxide Assay

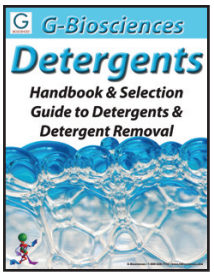
- Lysis Buffers & Systems
- Protein Fractionation Kits
- Dialysis (Micro) System
- Electrophoresis Clean-Up
- Concentration Systems
- Contamination Removal

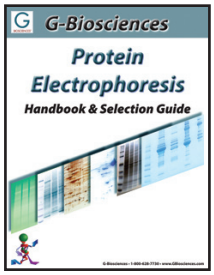




- Protease Inhibitor Cocktails
- Individual Protease Inhibitors
- Protease Assays
- Proteases for Mass Spec.
- Sequencing Grade Proteases

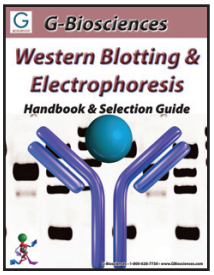
- Proteomic Grade Detergents
- Research Grade Detergents
- Non-Ionic, Ionic & Zwitterionic
- Detergent Estimations
- Detergent Removal Systems

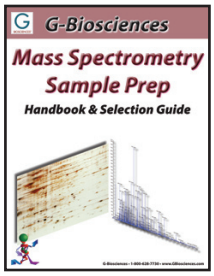




- Gel Preparation Chemicals
- Protein Marker Ladders
- Electrophoresis Buffers
- Reducing & Alkylating Reagents
- Protein Gel Stains

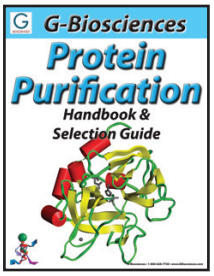
- 1-Hour Western System
- Transfer Buffers & Membranes
- Membrane Stains
- Blocking Buffers
- Secondary Antibodies
- Detection Reagents
- Reprobing Reagents

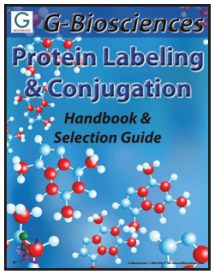




- Protein Sample Preparation
- Protein Clean-Up Systems
- Electrophoresis Reagents
- Mass Spec Grade Protease
- InGel Digestion Kits
- Peptide Generation Reagents

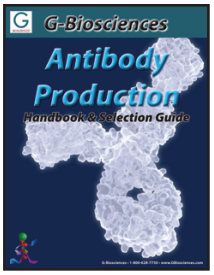
- Affinity Resins
- 6X His Protein Purification Kits
- GST Protein Purification Kits
- Antibody Purification
- Activated Resins
- Buffers & Reagents

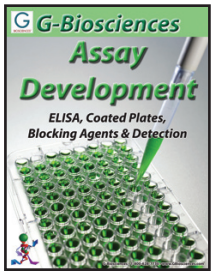




- Biotin Labeling
- Cell Surface Protein Labeling
- Agarose Coupling Kits
- Fluorescent Dye Labeling Kits
- Enzyme Labeling Systems

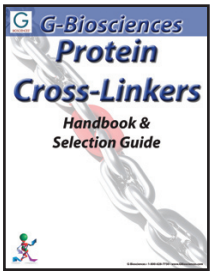
- Carrier Proteins
- Peptide Coupling Systems
- Antibody Purification Resins
- Antibody Fragmentation Kits

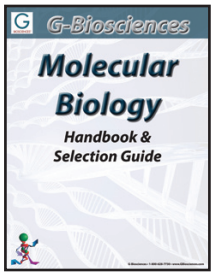




- Coated Plates
- Blocking Buffers
- Wash Buffers
- Secondary Antibodies
- Detection Reagents
- Antibody Labeling Systems

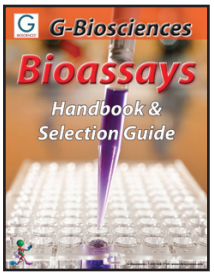
- Homobifunctional
- Heterobifunctional
- Optimizer Systems
- Cross-Linking Systems





- 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



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STRATEGY™

Develop purification strategy for novel proteins

STRATEGY™ consists of numerous fractionation and chromatography techniques to aid researchers to develop a suitable purification system for specific and novel proteins.

A major issue in purifying a novel protein is that the characteristics of the protein to be purified are often unknown. Purification techniques, such as ion exchange and hydrophobic chromatography, rely on the knowledge that the protein of interest is charged or hydrophobic. A second issue is that protein purification rarely utilizes a single purification technique. When a researcher begins developing a purification strategy they often have to invest in large volumes of resins and buffers that may be ineffective in purification, resulting in a waste of money and time.

STRATEGY™ is designed to allow researchers to rapidly screen a number of purification techniques in a small scale format to identify the best system to isolate their protein of interest. The self contained kit allows researchers to save time and money as separate buffers, resins and columns are not needed.

The kit guides the researcher step-by-step through various protein purification techniques, which include:

- pH Fractionation
- Salt Fractionation
- Hydrophobic Chromatography
- Anionic Chromatography
- Cationic Chromatography

In addition, STRATEGY™ is supplied with reagents needed to clean up samples prior to analysis. No expensive equipment is needed.

After optimizing the purification strategy, the agents and supplies used in the kit may be ordered separately.

Cat. No.	Description	Size
786-184	STRATEGY™	1 kit
786-184A	Anionic Resin Columns	6 columns
786-184C	Cationic Resin Columns	6 columns
786-184HP	Phenyl HP Columns	6 columns

G-CAPSULE™

Electroelution device for the rapid purification of nucleic acids from electrophoresis gels

Electroelution of nucleic acids and proteins has many advantages as it avoids centrifugation, vortexing, heating, precipitation and allows minimal manipulation of samples. Electroelution normally involves dialysis tubing, which results in extreme dilution of precious samples. G-Capsule™ is a simple electroelution device that excises DNA or protein bands and elutes your sample in a final volume of ~30µl.

G-CAPSULE™ has two parts, G-Pick™ and G-Trap™. The user simply picks up the protein or nucleic acid band with the G-Pick™ and assembles it with the G-Trap™. The assembled G-CAPSULE™ is submerged in electrophoresis buffer on a horizontal electrophoresis system and the protein or nucleic acid is rapidly eluted into the G-Trap™.

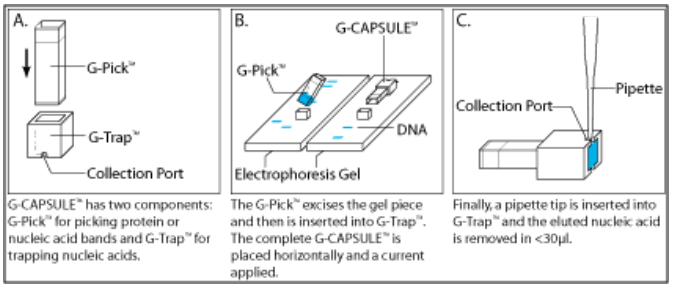


Figure 1: A schematic of the G-CAPSULE™ procedure.

FEATURES

- Rapid electroelution of nucleic acids and proteins
- Sample recovered in a small volume (25-50µl)
- Recovery is as high as 90%

APPLICATIONS

- G-CAPSULE™ can be used for extraction of >20bp DNA and RNA or for >4kDa proteins

ACCESSORIES

- G-CAPSULE™ Weight: A small weight device that prevents G-CAPSULE™ from floating during electroelution

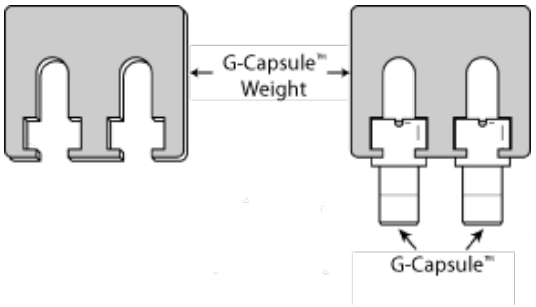


Figure 2: The G-CAPSULE™ weight.

CITED REFERENCES

Chatterjee, S et al (2012) Acta Biochim Biophys Sin. 259:68
Chatterjee, S et al (2012) Acta Biochim Biophys Sin. 44:259
Chatterjee, S et al (2012) Acta Biochim Biophys Sin. 10:1093.
Cardi, D et al (2010) J. Biol. Chem. 285:26406-26416.
Crosslin, James (2009) HortScience. 44: 1790 - 1791
Li, X. et al (2004) Euro J of Phycology. 39: 1, 73-82
Beeson, K. et al (2002) Microbiology. 148: 179 - 189
Yeager, M. et al (2001) Circ. Res. 88: 2e - 11
Robu, M. et al (2001) PNAS. 98: 8211
Dudley, E. et al (2001) Microbiology. 147: 215 - 224
Brezinschek, Hans-Peter et al (2000) Int. Immunol. 12: 767

Cat. No.	Description	Size
786-001	G-CAPSULE™	55/box
786-004	G-CAPSULE™ Weight	1 weight

Activated resins have immobilized groups bound to agarose beads that can be used to generate specific affinity columns for protein, antibody and other molecule purification.

Activated resins offered include:

- Sulfhydryl Coupling Resin: Activated iodoacetyl groups for coupling free sulfhydryls
- Amine Coupling Resin: Activated aldehyde groups for coupling primary amines
- CDI Amine Reacitve: Reactive imidazole carbamates to couple primary amines. Ideal for peptide immobilization
- Carboxyl Coupling Resin: Immobilized DADPA (Diaminodipropylamine) generates a free amine to conjugate carboxyls and other moieties with the aid of crosslinkers
- Carbohydrate Coupling Resin: Hydrazide activated agarose for coupling of oxidized carbohydrates
- SDC™ Immobilization: Uses Immobilized DADPA (Diaminodipropylamine)) for the immobilization of steroids, drugs and chemical compounds that lack primary amines, sulfhydryls, carbonyls and other common coupling groups

SULFHYDRYL REACTIVE

Sulfhydryl Coupling Resin

Activated iodoacetyl group for binding free sulfhydryls

The Sulfhydryl Coupling Resin is designed for the simple and efficient coupling of peptides and proteins to a solid 6% agarose support through free sulfhydryl groups (-SH). The iodoacetyl groups of the Sulfhydryl Coupling Resin specifically react with free sulfhydryls to form covalent, permanent thioether bonds. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

The Sulfhydryl Coupling Resin is available as a resin slurry or prealiquoted as five 2ml spin column format.

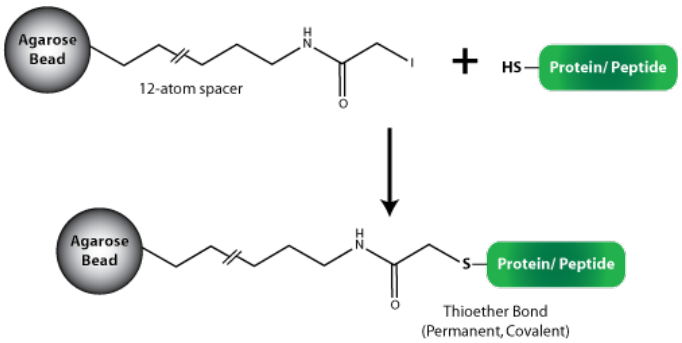


Figure 3: Sulfhydryl Coupling Resin scheme.

FEATURES

- Stable coupling of proteins and peptides, forms covalent thioether bonds
- Couples 1-2mg peptide and 2-20mg protein/ml resin

APPLICATIONS

- For the generation of affinity columns for antibody purification and other affinity chromatography

Cat. No.	Description	Size
786-794	Sulfhydryl Coupling Resin	10ml resin
786-795	Sulfhydryl Coupling Resin	50ml resin
786-796	Sulfhydryl Coupling Resin	250ml resin
786-806	Sulfhydryl Coupling Resin	5 x 2ml columns

Sulfhydryl Immobilization Kit for Proteins

For generation of protein affinity columns through free sulfhydryls

The Sulfhydryl Immobilization Kit for Proteins is a complete kit designed for the simple and efficient coupling of proteins to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that specifically react with free sulfhydryls to form covalent, permanent thioether bonds. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

Proteins, including antibodies, must have free sulfhydryls for immobilization to the resin. A mild reducing agent, 2-Mercaptoethylamine, is supplied to reduce the hinge region disulfide bonds of antibodies, while preserving the functionally crucial disulfide bonds between the heavy and light chains.

The resulting columns can be used to study protein-protein interactions or for purification, via affinity chromatography. The columns, depending on the stability of the immobilized molecule, can be used several times without significant loss of activity.

FEATURES

- Generates 5 reusable, spin format affinity columns
- Specific conjugation through free sulfhydryls
- High Capacity: 2-40mg protein/ column
- Supplied with mild reducing agent for free sulfhydryls generation

APPLICATIONS

- Immobilize proteins to purify interacting molecules
- Immobilize antibodies in the correct orientation

Cat. No.	Description	Size
786-804	Sulfhydryl Immobilization Kit for Proteins	For 5 x 2ml columns

Sulfhydryl Immobilization Kit for Peptides

For generation of peptide affinity columns through free sulfhydryls

Sulfhydryl Immobilization Kit for Peptides is designed for the simple and efficient coupling of sulfhydryl-containing peptides to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that specifically react with free sulfhydryls to form covalent, permanent thioether bonds. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

Peptides must have free sulfhydryls for immobilization to the resin. The supplied Protein-S-S-Reductant™ reducing agent efficiently reduces disulfide bonds and does not interfere with the iodoacetyl coupling reaction. Protein-S-S-Reductant™ offers the advantage that it does not require removal before peptide immobilization.

The resulting columns can be used for the purification of antibodies that have been raised against the specific peptide. The columns, depending on peptide stability, can be used several times.

FEATURES

- Generates 5 reusable, spin format affinity columns
- Specific conjugation through free sulfhydryls
- High Capacity: 2-4mg peptide/column

APPLICATIONS

- Immobilize peptides for antibody purification

Cat. No.	Description	Size
786-805	Sulfhydryl Immobilization Kit for Peptides	For 5 x 2ml columns

AMINE REACTIVE

Amine Coupling Resin

The amine reactive HOOK™ Activated Agarose is 6% agarose that has been activated to generate reactive aldehyde groups. The aldehyde groups of the agarose react spontaneously 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 reductive amination to form stable amine linkages between the agarose and the ligand.

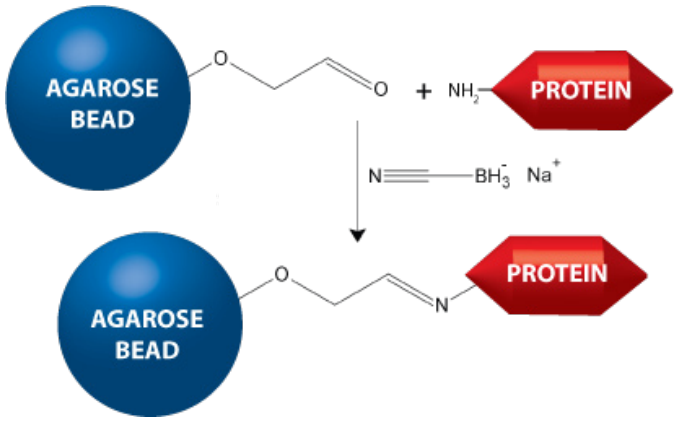


Figure 4: Scheme for the coupling of proteins to HOOK™ Activated Agarose (Amine Reactive).

The amine reactive HOOK™ Activated agarose is also supplied in a complete kit for the generation of 5 x 2ml resins. The kit is supplied with all the necessary reagents and columns.

FEATURES

- Binding capacity: 20mg protein/ml resin
- 6% cross-linked agarose

APPLICATIONS

- Coupling of proteins and peptides to agarose beads
- Suitable for antibody purification

CITED REFERENCES

Rudolph, V. et al (2008) J. Pharmacol. Exp. Ther. 327:324

Cat. No.	Description	Size
786-066	HOOK™ Activated Agarose (Amine Reactive)	10ml resin
786-063	HOOK™ Activated Agarose Coupling Kit (Amine Reactive)	For 5 x 2ml columns

CDI Amine Reactive Resin

G-Biosciences CDI Amine Reactive Agarose consists of 6% cross-linked agarose activated with CDI (1,1'-carbonyl diimidazole) to form reactive imidazole carbamates.

The activation of the resin occurs in solvent and to maintain its activity the resin is supplied in acetone to prevent hydrolysis. Upon reaction of the resin with primary amine containing molecules, i.e. proteins, in basic (pH8.5-10) aqueous buffers the imidazole carbamates lose the imidazole group and form carbamate linkages.

CDI Amine Reactive Agarose is ideal for immobilizing peptides, small organic molecules and certain proteins and reactions can occur in organic solvent making it ideal for water-insoluble ligands.

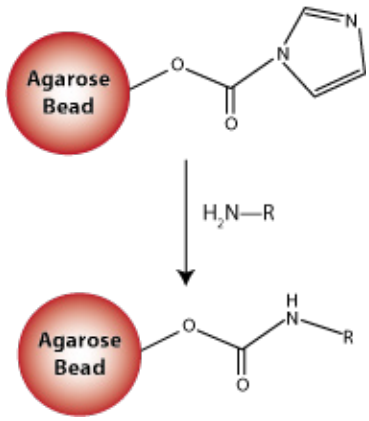


Figure 5: Scheme for the coupling of proteins to CDI Amine Reactive Agarose.

FEATURES

- Proven coupling chemistry
- Easy to use, no secondary coupling agents required
- Stable linkages
- Couple in inorganic buffers for insoluble molecules

APPLICATIONS

- Couple proteins and peptides
- Couple primary amine containing ligands

Cat. No.	Description	Size
786-404	CDI Amine Reactive Resin	10ml resin

CARBOXYL REACTIVE

Carboxyl Coupling Resin

Consists of 6% cross-linked agarose with covalent linked diaminodipropylamine (DADPA) to generate a free primary amine at the end of a long spacer arm.

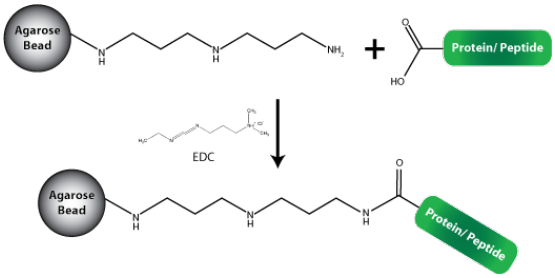


Figure 6: Carboxyl Coupling Resin scheme.

Molecules, including proteins and peptides, are covalently coupled to the free primary amines, and the stable columns are ideal for affinity purification of antibodies and other interacting partners. Molecules can be coupled to the free amine by numerous amine-reactive methods; however the use of the carbodiimide EDC allows coupling of free carboxyl groups. The resulting amide bond is highly stable and greatly reduces the chance of leaching of the affinity tag. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

FEATURES

- Immobilized DADPA (diaminodipropylamine)
- 6% cross-linked agarose
- Long spacer arm to limit steric hindrance

APPLICATIONS

- Couple peptides for antibody purification
- Couple peptides and proteins to purify interacting molecules

Cat. NO.	Description	Size
786-797	Carboxyl Coupling Resin (Immobilized DADPA (Diaminodipropylamine))	25ml resin

CARBOHYDRATE REACTIVE

Carbohydrate Coupling Resin

Immobilize glycoproteins through carbohydrates.

For the covalent immobilization of carbohydrate containing molecules, including glycoproteins, to agarose beads.

Carbohydrate-containing molecules are treated with sodium meta-periodate to oxidize their cis-diol groups to aldehydes. The aldehydes spontaneously react with the hydrazide goups on the agarose beads to form stable covalent bonds. The stable nature allows the affinity resin to be used multiple times.

Ideal for the coupling of polyclonal antibodies as it allows for the optimal orientation of the antibodies for affinity purification.

The Carbohydrate Coupling kit includes 5 x 2ml Carbohydrate Coupling spin columns, SpinOUT™ desalting columns and sodium meta-periodate.

FEATURES

- Hydrazide activated agarose
- Capacity: 1-5mg antibody/ml resin

Cat. No.	Description	Size
786-807	Carbohydrate Coupling Kit	For 5 columns
786-808	Carbohydrate Coupling Resin	5ml resin

ACTIVE HYDROGEN REACTIVE

SDC™ (Steroid/Drug/Compound) Immobilization

Designed for the immobilization of steroids, drugs and chemical compounds that lack primary amines, sulfhydryls, carbonyls and other common coupling groups to a solid-phase agarose support for the use in affinity purification. The kit uses Immobilized DADPA (diaminodipropylamine) resin to bind steroids, drugs and chemicals through their active hydrogens.

The coupling uses the Mannich reaction, which is described as the condensation of formaldehyde with ammonia, in the form of its salt, and another compound containing an active hydrogen. The SDC™ Immobilization kit replaces the ammonia with the primary amine on the DADPA and the active hydrogen is supplied by the steroid, drug or chemical to be coupled. Ideal for the generation of five 2ml affinity columns.

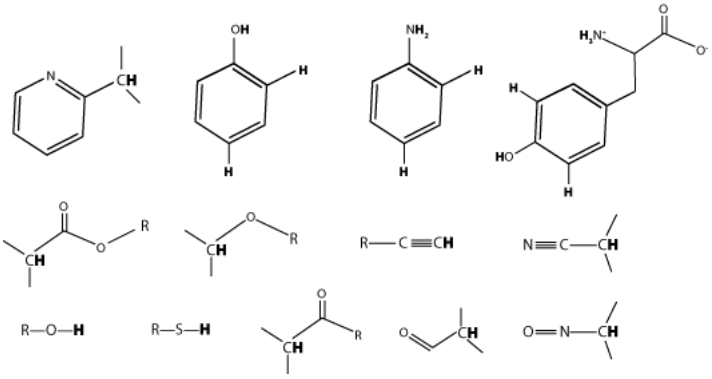


Figure 7: Active hydrogen containing compounds.

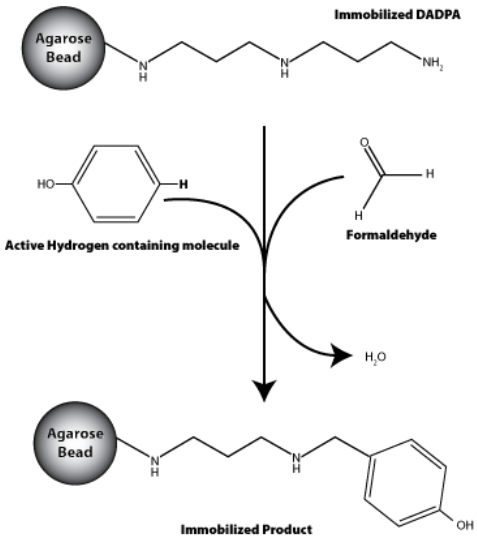


Figure 8: SDC™ (Steroid/ Drug/ Compound) Immobilization scheme.

FEATURES

- Uses Immobilized DADPA (diaminodipropylamine) resin
- Stable, covalent linkage

APPLICATIONS

- Immobilization of drugs, steroids and small metabolites through active hydrogens
- Ideal for compounds lacking primary amines, sulfhydryls, carbonyls and other common coupling groups

Cat. NO.	Description	Size
786-271	SDC™ (Steroid/Drug/Compound) Immobilization	5 reactions

CBP PURIFICATION

Calmodulin Resin

Calmodulin Resin for the affinity purification of calmodulin binding proteins (CBP), including recombinant proteins with a CBP tag and calmodulin-regulated proteins in eukaryotic cells. The resin is 4% agarose coupled to calmodulin and has a ligand density of approximately 1mg calmodulin/ml resin.

The Calmodulin Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Calmodulin Binding/Wash Buffer (50mM Tris-HCl (pH7.5), 200mM NaCl, 2mM $CaCl_2$)
- 100ml Calmodulin Elution Buffer (50mM Tris-HCl (pH7.5), 200mM NaCl, 2mM EGTA)
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the purification of calmodulin binding proteins
- Binds CBP tagged recombinant proteins
- High capacity: ~1-3mg/ml
- Bead size: 50-160µm
- Bead Structure: 4% highly cross-linked agarose
- Ligand density: 0.9-1.2mg calmodulin/ml resin

APPLICATIONS

- Affinity purification of proteins with a calmodulin binding protein (CBP) motif.

Cat. No.	Description	Size
786-282	Calmodulin Resin	10ml resin
786-552	Calmodulin Resin Kit	1
786-546	Calmodulin Binding/ Wash Buffer	100ml
786-547	Calmodulin Elution Buffer	100ml

GST PURIFICATION

Glutathione Resin

For the isolation of GST recombinant proteins

Designed for the affinity purification of proteins with a glutathione S-transferase (GST) tag. The resin consists of reduced glutathione (GSH) covalently coupled to 4% cross-linked agarose, via a 10-carbon spacer arm. The resin has a binding capacity of ~8mg GST/ml resin. Supplied as slurry in 20% ethanol.

Glutathione Resin is available as resin alone or supplied in a kit format.

FEATURES

- For the purification of GST proteins
- High capacity (~8mg/ml)
- Ligand density: 7-15µmoles glutathione/ml
- Bead size: 50-160µm
- Bead structure: 4% cross-linked agarose
- 10 carbon spacer arm

Cat. No.	Description	Size
786-280	Glutathione Resin	12.5ml resin
786-310	Glutathione Resin	25ml resin
786-311	Glutathione Resin	100ml resin
786-312	Glutathione Resin	500ml resin
786-540	GST Binding/ Wash Buffer	100ml
786-541	GST Elution Buffer	100ml

HOOK™ GST Protein Purification (Bacteria)

Isolate GST recombinant proteins from bacteria

The bacteria are first lysed with Bacterial PE LB™ and PE LB™-Lysozyme to release total soluble protein, whilst maintaining the structure and activity of the protein. The GST tagged protein is purified by passing clarified lysate through prepacked columns.

Bacterial- PELB™ kit has been developed for the extraction of soluble proteins from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial-PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force.

The kit is optimized to yield up to 10mg/250ml culture of soluble GST tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein. For small scale, rapid GST protein isolation from bacteria we recommend our spin format kits.

FEATURES

- Isolate >10mg GST tagged proteins from bacterial cultures
- No sonication required, supplied with lysis buffer and enzyme
- Suitable for 5 isolations from 250ml bacterial culture

Cat. No.	Description	Size
786-641	HOOK™ GST Protein Purification Kit (Bacteria)	5

HOOK™ GST Protein Spin Purification (Bacteria)

Spin format isolation of GST recombinant protein from bacteria

The bacteria are first lysed with Bacterial PE LB™ and PE LB™-Lysozyme to release total soluble protein, whilst maintaining the structure and activity of the protein. The GST tagged protein is purified by affinity chromatography by adding 0.5ml glutathione resin to the clarified lysate. The resin is transferred to a convenient spin column, where it is rapidly washed and the GST protein is eluted.

Bacterial- PELB™ kit has been developed for the extraction of soluble proteins from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial-PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force.

HOOK™ GST Protein Spin Purification kit is optimized to yield ~1mg/50ml culture of soluble GST tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

For larger scale GST protein isolation from bacteria we recommend our gravity flow kits for >10mg protein from ~250ml bacteria.

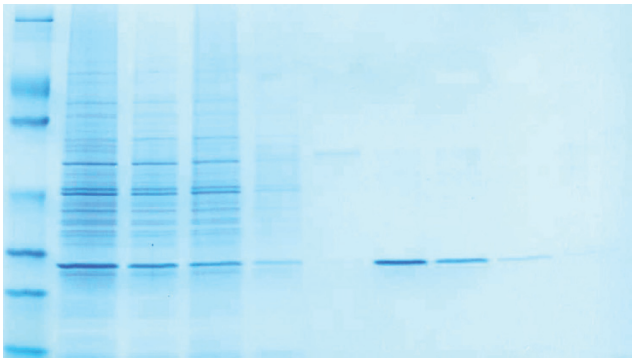


Figure 9: Bacteria expressing a GST-tagged protein were lysed with Bacterial PE-LB™ and the recombinant protein was purified with HOOK™ GST Protein Spin Purification kit. Lane 1: PAGEmark™ protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

- Isolate >1mg GST tagged proteins from ~50ml bacterial culture
- No sonication required, supplied with lysis buffer and enzyme
- Spin column format
- Suitable for 25 isolations from 50ml culture

Cat. No.	Description	Size
786-640	HOOK™ GST Protein Spin Purification Kit (Bacteria)	25

HOOK™ GST Protein Purification (Yeast)

Isolate GST recombinant proteins from yeast

The yeast are first lysed with Yeast PE LB™ and LongLife™ Zymolyase® to release total soluble protein, whilst maintaining the structure and activity of the protein. The GST tagged protein is purified by passing the lysate through prepacked columns.

Yeast-PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided.

Optimized to yield up to 10mg of soluble GST tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

FEATURES

- Isolate >10mg GST tagged proteins from bacterial cultures
- No sonication required, supplied with lysis buffer and enzyme
- Suitable for 5 isolations from 1ml yeast cell pellet

Cat. No.	Description	Size
786-643	HOOK™ GST Protein Purification Kit (Yeast)	5

HOOK™ GST Protein Spin Purification (Yeast)

Spin format isolation of GST proteins from yeast

HOOK™ GST Protein Spin Purification kit allows for the rapid purification of soluble, GST tagged protein from yeast cultures. The yeast are first lysed with Yeast PE LB™ and LongLife™ Zymolyase® to release total soluble protein, whilst maintaining the structure and activity of the protein. The GST tagged protein is purified by affinity chromatography by adding 0.5ml Glutathione resin to the clarified lysate. The resin is transferred to a convenient spin column, where it is rapidly washed and the GST protein is eluted.

Yeast-PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided. Depending on application, additional agents such as protease inhibitors may be added into Yeast PE LB™. The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast PE LB™ eliminates the need for laborious glass bead lysis of yeast cells.

Optimized to yield ~1mg of soluble GST tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

For larger scale GST protein isolation from yeast we recommend our gravity flow kits for >10mg protein from yeast.

FEATURES

- Isolate >1mg GST tagged proteins from yeast
- No sonication required, supplied with lysis buffer and enzyme
- Spin column format
- Suitable for 25 isolations from <0.5ml yeast cell pellet

Cat. No.	Description	Size
786-642	HOOK™ GST Protein Spin Purification Kit (Yeast)	25

IMAC PURIFICATION

Immobilized metal affinity chromatography resins for 6X His tagged protein purification

A large selection of resins and kits for the isolation of His tagged recombinant proteins are available.

Four different IMAC purification resins are offered:

NICKEL CHELATING RESIN

The most commonly used IMAC purification resin for the purification of 6X His recombinant proteins that offers high binding efficiency and low non-specific binding.

COBALT CHELATING RESIN

Growing increasingly popular due to its advantage over Nickel Chelating Resin. Although 6X His recombinant proteins bind with a slightly lower efficiency compared to Nickel Chelating Resin there is a significant reduction in non-specific binding. Cobalt resins have a higher selectivity for poly-His sequences, however have a low loading capacity, therefore Cobalt Chelating Resin should be used for valuable recombinant proteins in limited quantities.

ZINC CHELATING RESIN

For the purification on zinc binding proteins, including 6x His recombinant proteins. Available as a resin.

COPPER CHELATING RESIN

For the purification on copper binding proteins, including 6x His recombinant proteins. Available as a resin.

Cobalt has the highest selectivity of the resins followed by Zinc, Nickel then Copper, but has the lowest loading capacity. Copper has the highest loading capacity, followed by Nickel then Zinc.

Nickel Chelating Resin

For the isolation of 6X His recombinant proteins

Immobilized metal affinity chromatography (IMAC) resin utilizing nickel (Ni^{2+}) for 6X histidine tagged protein purification.

This resin binds to six histidine residues (6X His), a common tag used in protein purification. The resin consists of iminodiacetate coupled to 6% cross-linked agarose beads. The iminodiacetate binds divalent nickel ion with a capacity of 20-40µmoles Ni^{2+}/ml resin.

The Nickel Chelating Resin is available as resin alone or in a spin column format:

Specific binding and elution buffers are also available:

- 100ml His Binding/Wash Buffer
  (10mM Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))
- 100ml His Elution Buffer
  (0.25M Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))

FEATURES

- For the purification of 6X His proteins
- High capacity: >50mg/ml
- Ligand density: 20-40µmoles Ni^{2+}/ml resin
- Bead Structure: 6% cross-linked agarose

CITED REFERENCES

RShukla, S et al (2011). Eukaryot. Cell. 10:1357
Shukla, S. et al (2011). Eukaryot. Cell. 10:1357

Cat. No.	Description	Size
786-281	Nickel Chelating Resin	12.5ml resin
786-407	Nickel Chelating Resin	100ml resin
786-408	Nickel Chelating Resin	500ml resin
786-542	His Binding/Wash Buffer	100ml
786-543	His Elution Buffer	100ml

Cobalt Chelating Resin

For the isolation of 6X His recombinant proteins

Specifically designed for the purification of proteins that associate with Cobalt ions, including 6X histidine tagged proteins. Although 6X His tagged proteins bind with a slightly lower efficiency compared to Nickel Chelating Resin there is a significant reduction in non-specific binding. Cobalt resins have a higher selectivity for poly-His sequences, however have a low loading capacity, therefore Cobalt Chelating Resin should be used for valuable recombinant proteins in limited quantities.

The resin consists of iminodiacetate coupled to 6% cross-linked agarose beads, which binds divalent cobalt ion with a capacity of 20-40µmoles Co^{2+}/ml resin. The protein binding capacity is >50mg protein per ml resin.

Specific binding/wash and elution buffers are available:

- 100ml His Binding/Wash Buffer
  (10mM Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))
- 100ml His Elution Buffer
  (0.25M Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))

FEATURES

- For the purification of 6X His proteins
- High capacity: >50mg/ml
- Ligand density: 20-40µmoles Co^{2+}/ml resin
- Bead Structure: 6% cross-linked agarose

Cat. No.	Description	Size
786-286	Cobalt Chelating Resin	12.5ml resin
786-402	Cobalt Chelating Resin	100ml resin
786-403	Cobalt Chelating Resin	500ml resin

Nickel & Cobalt Resin Spin Columns

For the isolation of 6X His recombinant proteins

In addition to the above resins, the Nickel and Cobalt Chelating Resins are supplied in prepackaged spin columns. Spin columns with resin bed volumes of 0.2, 1 and 3ml are available. The total column volumes are 1, 8 and 22ml respectively.

Specifically designed for the purification of proteins that associate with Nickel or Cobalt ions, including 6X histidine tagged proteins. Although 6X His tagged proteins bind with a slightly lower efficiency compared to Nickel Chelating Resin there is a significant reduction in non-specific binding. Cobalt resins have a higher selectivity for poly-His sequences, however have a low loading capacity, therefore Cobalt Chelating Resin should be used for valuable recombinant proteins in limited quantities.

The resin consists of iminodiacetate coupled to 6% cross-linked agarose beads, which binds divalent cobalt ion with a capacity of 20-40µmoles Co^{2+}/ml resin. The protein binding capacity is >50mg protein per ml resin.

FEATURES

- For the purification of 6X His proteins
- Available as 0.2, 1 and 3ml resin
- High capacity: >50mg/ml
- Ligand density: 20-40µmoles Ni^{2+} or Co^{2+}/ml resin
- Bead Structure: 6% cross-linked agarose

Cat. No.	Description	Size
786-392	Nickel Chelating Resin, 0.2ml Spin Column	25 columns
786-393	Nickel Chelating Resin, 1ml Spin Column	5 columns
786-394	Nickel Chelating Resin, 3ml Spin Column	5 columns
786-454	Cobalt Chelating Resin, 0.2ml Spin Column	25 columns
786-455	Cobalt Chelating Resin, 1ml Spin Column	5 columns
786-456	Cobalt Chelating Resin, 3ml Spin Column	5 columns

Copper Chelating Resin
Zinc Chelating Resin

For the isolation of 6X His recombinant proteins

Specifically designed for the purification of proteins that associate with copper or zinc ions, including 6X histidine tagged proteins.

The resin consists of iminodiacetate coupled to 6% cross-linked agarose beads, which binds divalent copper ion with a capacity of 20-40µmoles Cu^{2+} or Zn^{2+}/ml resin. The protein binding capacity is >50mg protein per ml resin.

Specific binding/wash and elution buffers are available:

- 100ml His Binding/Wash Buffer (10mM Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))
- 100ml His Elution Buffer (0.25M Imidazole, 0.3M NaCl, 0.05M sodium phosphate (pH8.0))

FEATURES

- Purification of copper or zinc binding proteins, including 6x His proteins
- High capacity: >50mg/ml
- Ligand density: 20-40µmoles Cu^{2+} or Zn^{2+}/ml resin
- Bead Structure: 6% cross-linked agarose

Cat. No.	Description	Size
786-285	Copper Chelating Resin	12.5ml resin
786-287	Zinc Chelating Resin	12.5ml resin
786-542	His Binding/Wash Buffer	100ml
786-543	His Elution Buffer	100ml

HOOK™ 6X His Protein Purification (Bacteria)

Complete kit for the isolation of 6X His recombinant proteins from bacteria

For the purification of soluble, 6X His tagged protein from bacterial cultures. The bacteria are first lysed with Bacterial PE LB™ and PE LB™-Lysozyme to release total soluble protein, whilst maintaining the structure and activity of the protein. The 6X His tagged protein is purified by immobilized metal affinity chromatography (IMAC) by passing clarified lysate through prepacked columns.

Bacterial- PELB™ has been developed for the extraction of soluble proteins from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial-PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force.

The kit is available with either nickel chelating immobilized metal affinity chromatography (IMAC) columns or cobalt chelating IMAC columns. Cobalt chelating resin has a lower binding affinity for 6X His tags, compared to nickel chelating resin, which results in less non-specific binding and may result in slightly lower yields.

Optimized to yield >10mg/250ml culture of soluble His tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

FEATURES

- Isolate >10mg His tagged proteins from ~250ml bacteria
- No sonication required, supplied with lysis buffer and enzyme
- Nickel or Cobalt resins available
- Suitable for 5 isolations from 250ml culture

Cat. No.	Description	Size
786-630	HOOK™ 6X His Protein Purification (Bacteria): Nickel Resin	5
786-631	HOOK™ 6X His Protein Purification (Bacteria): Cobalt Resin	5

HOOK™ 6X His Protein Spin Purification (Bacteria)

Complete spin format kit for the isolation of 6X His recombinant proteins from bacteria

HOOK™ 6X His Protein Spin Purification kit allows for the rapid purification of soluble, 6X His tagged protein from bacterial cultures.

The bacteria are first lysed with Bacterial PE LB™ and PE LB™-Lysozyme to release total soluble protein, whilst maintaining the structure and activity of the protein. The 6X His tagged protein is purified by immobilized metal affinity chromatography (IMAC) by adding 0.5ml immobilized metal affinity resin to the clarified lysate. The resin is transferred to a convenient spin column, where it is rapidly washed and the 6X His protein is eluted with an imidazole buffer.

Bacterial- PELB™ has been developed for the extraction of soluble proteins from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial-PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force. No sonication required.

HOOK™ 6X His Protein Spin Purification kit is available with either nickel chelating resin or cobalt chelating resin for the immobilized metal affinity chromatography. Cobalt chelating resin has a lower binding affinity for 6X His tags, compared to nickel chelating resin, which results in less non-specific binding and may result in slightly lower yields.

HOOK™ 6X His Protein Spin Purification kit is optimized to yield ~1mg/50ml culture of soluble His tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

For larger scale His protein isolation from bacteria we recommend our gravity flow kits for >10mg protein from ~250ml bacteria.

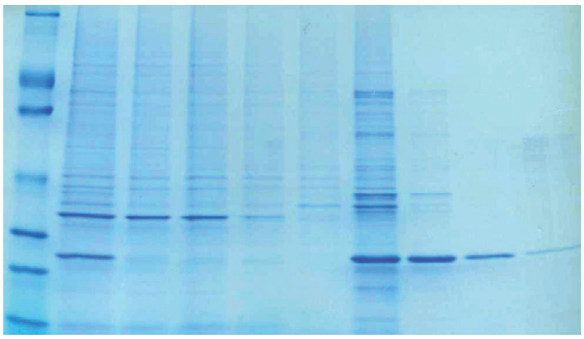


Figure 10: Bacteria expressing a 6X His-tagged protein were lysed with Bacterial PE-LB™ and the recombinant protein was purified with HOOK™ 6X His Protein Spin Purification kit. Lane 1: PAGEmark™ protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

- Isolate >1mg His tagged proteins from ~50ml bacterial culture
- No sonication required, supplied with lysis buffer and enzyme
- Nickel or Cobalt resins available
- Spin column format
- Suitable for 25 isolations from 50ml culture

APPLICATIONS

- Complete kit for the spin format isolation of 6X His tagged recombinant proteins from bacteria

Cat. No.	Description	Size
786-628	HOOK™ 6X His Protein Spin Purification Kit (Bacteria): Nickel Resin	25
786-629	HOOK™ 6X His Protein Spin Purification Kit (Bacteria): Cobalt Resin	25

HOOK™ 6X His Protein Purification (Yeast)

Complete kit for the isolation of 6X His recombinant proteins from yeast

HOOK™ 6X His Protein Purification kit allows for the purification of soluble, 6X His tagged protein from yeast cultures. The yeast are first lysed with Yeast PE LB™ and LongLife™ Zymolyase® to release total soluble protein, whilst maintaining the structure and activity of the protein. The 6X His tagged protein is purified by immobilized metal affinity chromatography (IMAC) by passing the clarified lysate through prepacked columns.

Yeast-PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided. Depending on application, additional agents such as protease inhibitors may be added into Yeast PE LB™. The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast PE LB™ eliminates the need for laborious glass bead lysis of yeast cells.

HOOK™ 6X His Protein Purification kit is available with either nickel chelating immobilized metal affinity chromatography (IMAC) columns or cobalt chelating IMAC columns. Cobalt chelating resin has a lower binding affinity for 6X His tags, compared to nickel chelating resin, which results in less non-specific binding and may result in slightly lower yields.

HOOK™ 6X His Protein Spin Purification kit is optimized to yield >10mg of soluble His tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

For small scale, rapid His protein isolation from yeast we recommend our spin format kits.

FEATURES

- Isolate >10mg His tagged proteins from yeast cultures
- No sonication required, supplied with lysis buffer and enzyme
- Nickel or Cobalt resins available
- Suitable for 5 isolations from 1ml yeast cell pellet

APPLICATIONS

- Complete kit for the isolation of 6X His tagged recombinant proteins from yeast

Cat. No.	Description	Size
786-634	HOOK™ 6X His Protein Purification (Yeast): Nickel Resin	5
786-635	HOOK™ 6X His Protein Purification (Yeast): Cobalt Resin	5

HOOK™ 6X His Protein Spin Purification (Yeast)

Complete spin format kit for the isolation of 6X His recombinant proteins from yeast

HOOK™ 6X His Protein Spin Purification kit allows for the rapid purification of soluble, 6X His tagged protein from yeast cultures. The yeast are first lysed with Yeast PE LB™ and LongLife™ Zymolyase® to release total soluble protein, whilst maintaining the structure and activity of the protein. The 6X His tagged protein is purified by immobilized metal affinity chromatography (IMAC) by adding 0.5ml immobilized metal affinity resin to the clarified lysate. The resin is transferred to a convenient spin column, where it is rapidly washed and the 6X His protein is eluted with an imidazole buffer.

Yeast-PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided. Depending on application, additional agents such as protease inhibitors may be added into Yeast PE LB™. The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast PE LB™ eliminates the need for laborious glass bead lysis of yeast cells.

HOOK™ 6X His Protein Spin Purification kit is available with either nickel chelating resin or cobalt chelating resin for the immobilized metal affinity chromatography. Cobalt chelating resin has a lower binding affinity for 6X His tags, compared to nickel chelating resin, which results in less non-specific binding and may result in slightly lower yields.

HOOK™ 6X His Protein Spin Purification kit is optimized to yield ~1mg of soluble His tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

For larger scale His protein isolation from yeast we recommend our gravity flow kits for >10mg protein from yeast.

FEATURES

- Isolate >1mg His tagged proteins from yeast
- No sonication required, supplied with lysis buffer and enzyme
- Nickel or Cobalt resins available
- Spin column format
- Suitable for 25 isolations from <0.5ml yeast cell pellet

APPLICATIONS

- Complete kit for the spin format isolation of 6X His tagged recombinant proteins from yeast

Cat. No.	Description	Size
786-632	HOOK™ 6X His Protein Spin Purification (Yeast): Nickel Resin	25
786-633	HOOK™ 6X His Protein Spin Purification (Yeast): Cobalt Resin	25

Streptavidin Resin

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-hydrochloride at pH1.5 or by boiling in SDS Page Sample Loading Buffer.

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 is covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The Steptavidin Resin is designed for the single step small and large scale affinity purification of proteins and antibodies with a biotin tag. The resin can also be used for immunoprecipitations using biotin labeled antibodies. Supplied as a resin slurry or in a 1ml spin column format.

Specific Binding and Elution Buffers are also available.

The Streptavidin Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Streptavidin Binding/Wash Buffer (20mM $NaPO_4$, 0.15M NaCl, pH7.5)
- 100ml Streptavidin Elution Buffer (8M Guanidine.HCl pH1.5)
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- 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. No.	Description	Size
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
786-555	Streptavidin Resin Kit	1
786-548	Streptavidin Binding Buffer	100ml
786-549	Streptavidin Elution Buffer	100ml

Avidin Resin

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}). 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 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=$10^{15}M^{-1}$).

The avidin in covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The Avidin Resin is designed for the single step small and large scale affinity purification of proteins and antibodies with a biotin tag. The resin can also be used for immunoprecipitations using biotin labeled antibodies. Supplied as a 50% resin slurry.

Specific Binding and Elution Buffers are also available.

FEATURES

- Avidin covalently coupled to ~6% cross linked agarose. Minimal Leaching
- Binding capacity 15-20µg biotin/ml resin

APPLICATIONS

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

Cat. No.	Description	Size
786-593	Immobilized Avidin Resin	5ml resin
786-594	Immobilized Avidin Resin	25ml Resin
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. No.	Description	Size
786-595	Immobilized Monomeric Avidin	5ml resin
786-596	Immobilized Monomeric Avidin	10ml resin
786-597	Immobilized Monomeric Avidin	Kit

HOOK™ Cell Surface Protein Isolation

Complete cell surface protein labeling & isolation

Uses our proven biotin labeling and purification technology in conjunction with our Mammalian Cell PE LB™ lysis buffer to conveniently label cell surface proteins and isolate them for further analysis.

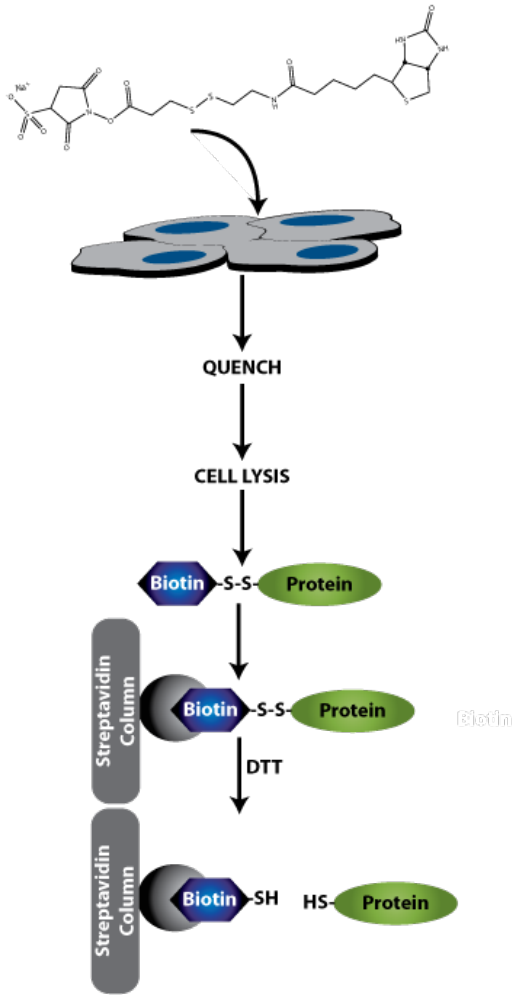


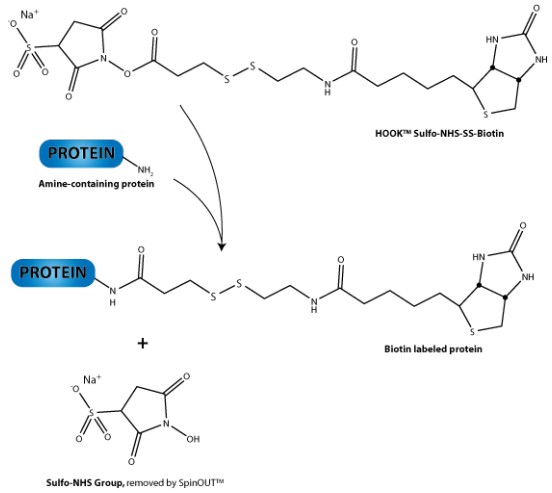
Figure 11: HOOK™ Cell Surface Protein Isolation scheme.

Mammalian cells, adherent or non-adherent, are labeled with Sulfo-NHS-SS-Biotin, an amine reactive biotinylation reagent that is soluble in water, but impermeable to plasma membranes. Sulfo-NHS-SS-Biotin has a disulfide bond in the spacer arm that permits the cleavage of the biotin moiety from the protein, making its interaction with streptavidin purification column reversible.

Molecular Weight	606.69
Spacer Arm (Å)	24.3
Reactive Group	sulfo-NHS ester
Membrane Permeable	NO
Water Soluble	YES
Cleavable/ Reversible	YES
Reaction pH	7-9

Table 1: Properties of HOOK™ sulfo-NHS-SS-Biotin.

Cells are lysed with Mammalian Cell PE LB™ and applied to a Streptavidin agarose column. Unlabeled intracellular proteins are washed away and the biotin labeled cell surface proteins are then released by reduction of the disulfide bond with DTT.



*Sulfo-NHS-SS-Biotin structure and coupling scheme.*The kit is supplied with all the necessary reagents and buffers for convenience and improved reproducibility. The kit is compatible with a wide variety of mammalian cells and can be used to compare treated and untreated cells and differences between different cell lines. This kit is supplied with sufficient reagents for five experiments, with each experiment consisting of four 90-95% confluent T-75cm^2 flasks.

FEATURES

- Complete cell surface labeling & isolation kit
- Convenient; all required reagents are included
- Versatile; suitable for wide selection of mammalian cells

APPLICATIONS

- For the isolation of cell surface proteins
- Study receptor:ligand interaction
- Study membrane trafficking

Cat. No.	Description	Size
786-316	HOOK™ Cell Surface Protein Isolation	5 Expts

Biotin Resin

Immobilized Biotin Resin is designed for the high affinity chromatography purifications of avidin, streptavidin and Neutravidin protein. The resin consists of biotin coupled to 6% cross-linked agarose.

Biotin, a 244 Dalton vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin (Ka=$10^{15}M^{-1}$) 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 boiling in SDS PAGE sample loading buffer.

FEATURES

- Strong affinity for avidin, streptavidin and neutravidin
- Reusable resin, at least 10 times
- Covalently coupled to limit leaching

APPLICATIONS

- Isolation of avidin, streptavidin and Neutravidin coupled molecules
- Immunoprecipitations with avidin, streptavidin and Neutravidin coupled antibodies

Cat. No.	Description	Size
786-598	Immobilized Biotin	5ml resin

Iminobiotin Resin

Immobilized Iminobiotin Resin consists of iminobiotin, a cyclic guanido analog of biotin, covalently coupled to 6% crosslinked agarose. The resin allows for the purification of avidin, streptavidin and Neutravidin and their subsequent gentle elution using non-denaturing elution buffers.

The normal biotin-avidin complex requires strong denaturing buffers, i.e. 8M guanidine • HCl, to denature the avidin and release the biotin, which obviously destroys the native and functional aspects of the avidin. The iminobiotin-avidin complex will form at >pH9.5 and can be dissociated at pH4.0 with gentle elution buffers, including 50mM ammonium acetate, pH4.0 with 0.5M NaCl.

FEATURES

- Biotin Binding Capacity: >2mg avidin/ml resin
- No requirement for strong, denaturing elution buffers
- Elutes at pH4.0

APPLICATIONS

- Isolation of avidin, streptavidin and Neutravidin complexes

Cat. No.	Description	Size
786-599	Immobilized Iminobiotin	5ml resin

BIOTIN CONJUGATION 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=35,500M^{-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 reduce the absorbance.

Cat. No.	Description	Size
BKC-01	HOOK™ BiotinQuant Kit	20 assays
BKC-03	HABA Dye	1g

Avidin

Affinity purified for biotin conjugation estimation

Avidin is a glycoprotein with approximately 10% of its total mass comes from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each have 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, temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications.This affinity purified avidin is ideal for estimation of biotin incorporation and other applications.

Cat. No.	Description	Size
786-581	Avidin	5mg
786-582	Avidin	25mg
786-583	Avidin	100mg

HABA

A biotin estimation dye reagent.

Cat. No.	Description	Size
BKC-03	HABA	1g

PROTEIN A & PROTEIN G

Immobilized Protein A

For binding the constant domains of immunoglobulin (Ig) molecules. Protein A is coupled to agarose beads by a proprietary coupling method that provides high coupling efficiency for immunoglobulins and minimal protein A leaching. Immobilized Protein A Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer *(0.1M sodium phosphate, 0.15M NaCl, pH7.5)*
- 100ml Protein A or G Elution Buffer *(100mM glycine, pH3.0)*
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: 18-43mg/ml resin
- Bead Structure: 6% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

CITED REFERENCES

Shi, L. et al (2009) J. Biol. Chem. 284: 3966 - 3975

Cat. No.	Description	Size
786-283	Immobilized Protein A Resin	5ml resin
786-553	Immobilized Protein A Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

Immobilized Protein G

For binding the constant domains of immunoglobulin (Ig) molecules. Protein G is a modified form of Streptococcal group G so that it does not bind to albumin. Protein G is coupled to 4% cross-linked agarose beads by a proprietary coupling method that provides high coupling efficiency for Ig and minimal protein G leaching. Immobilized Protein G Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer *(0.1M sodium phosphate, 0.15M NaCl, pH7.5)*
- 100ml Protein A or G Elution Buffer *(100mM glycine, pH3.0)*
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: >20mg Human IgG/ml resin
- Ligand density: ~2mg protein G /ml resin
- Bead size: 50-160µm
- Bead Structure: 4% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

Cat. No.	Description	Size
786-284	Immobilized Protein G Resin	5ml resin
786-554	Immobilized Protein G Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

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

PEARL™ PURIFICATION

Pearl™ IgG Purification Resin

For the one-step purifiication of the immunoglobulin G (IgG) antibodies from serum. The resin binds the high abundant, non-IgG proteins (i.e albumin) and allows the IgG molecules to pass through in a physiological buffer. The purified IgG molecules can be stored or used in further downstream applications without further clean-up, such as ammonium sulfate precipitation.

Purifies IgG in <15 minutes, which is more rapid than the commonly used Protein A and Protein G resins. The performance of the Pearl™ IgG Purification Resin is comparable or better than the Protein A and Protein G resins.

Species	Pearl™ IgG Purification Resin	Protein A	Protein G
Mouse	++++	++++	++++
Human	++++	++++	++++
Rat	++++	+	++
Hamster	++++	++	++
Guinea Pig	++++	++++	++
Rabbit	++++	++++	+++
Horse	++++	++	++++
Cow	++	++	++++
Pig	++++	+++	++
Sheep	++	+	++
Goat	++++	+	++
Chicken	-	-	-

Table 3: Binding efficiencies of Pearl™ IgG Purification Resin compared to Protein A and Protein G.

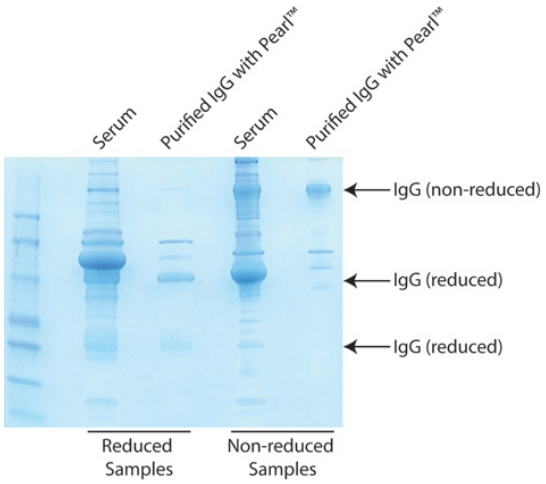


Figure 12: Pearl™ IgG Purification Resin rapidly purifies IgG molecules. Rabbit serum was dialyzed for 2 hours against IgG Purification Buffer and treated with IgG Purification Resin. The serum and flowthrough were compared under reducing and non reducing conditions.

FEATURES

- Simple 1-step purification
- High recovery (>90%) & Purity (>80%)

APPLICATIONS

- Purification of IgG (Immunoglobulin G) molecules
- Purify IgG from sources not compatible with Protein A & G

Cat. No.	Description	Size
786-800	Pearl™ IgG Purification Resin	3ml resin
786-801	Pearl™ IgG Purification Resin	25ml resin

Pearl™ IgG Purification Kits

Isolate IgG in physiological buffer in <15mins

The Pearl™ IgG Purification kits are designed for the one step purification of IgG (Immunoglobulin G) antibodies from serum. The supplied Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e albumin) and allows the IgG molecules to pass through in a physiological buffer. The purified IgG molecules can be stored or used in further downstream applications without further clean-up, such as ammonium sulfate precipitation.

The Pearl™ IgG purification kits can purify IgG in <15 minutes, which is more rapid than the commonly used Protein A and Protein G resins. The performance of the Pearl™ IgG Purification Resin is comparable or better than the Protein A and Protein G resins.

Pearl™ IgG Purification (Spin Format) kit is ideal for the rapid, small scale purification of IgG. The kit is supplied with 3ml Pearl™ IgG Purification Resin, IgG Isolation Buffer and 20 spin columns. Suitable for purifying up to 25mg IgG.

Pearl™ IgG Purification kit is supplied with 25ml Pearl™ IgG Purification Resin and IgG Isolation Buffer and is suitable for the isolation of IgG from ~100ml serum (~200mg IgG).

Cat. No.	Description	Size
786-798	Pearl™ IgG Purification (Spin Format) kit	For 25mg IgG
786-799	Pearl™ IgG Purification kit	For ~200mg IgG

Pearl™ Monoclonal IgG Purification

Isolate monoclonal antibodies from ascites & cell culture supernatant

The Pearl™ Monoclonal IgG Purification kit allows for the rapid purification of antibodies from cell culture supernatant and ascites fluid. The Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e. albumin) and allows the IgG molecules to pass through in a physiological buffer. The IgG molecules can be stored or used in downstream applications without further clean-up, such as ammonium sulfate precipitation.

The Pearl™ Monoclonal IgG Purification kit can be used to purify antibodies direct from cell culture supernatant with less than 10% FBS or can be used with ascites fluid after treatment with the supplied Ascites PreTreat.

The Pearl™ Monoclonal IgG Purification kit can purify IgG from ~1L cell culture supernatant or 200ml ascites fluid.

FEATURES

- Isolate monoclonal antibodies for ascites fluid or cell culture supernatant
- Supplied with ascites pretreatment reagent for optimal IgG purification
- For 1L of cell culture supernatant or 0.2L ascites fluid

APPLICATIONS

- Monoclonal antibody isolation from ascites fluid or cell culture supernatant

Cat. No.	Description	Size
786-802	Pearl™ Monoclonal IgG Purification Kit	1

Pearl™ Antibody Clean Up Kit

Removal of inhibitory BSA & gelatin from antibody solutons

Purified and commercial antibodies are routinely stored in buffers containing bovine serum albumin (BSA) and gelatin that act as stabilizers during long term storage. In routine applications, such as ELISA, Western blotting and other immunodetection techniques, these proteins generally do not interfere. The presence of the protein stabilizers do interfere with antibody labeling and conjugation techniques, including biotinylation, fluorescent dye labeling, covalent antibody immobilization and antibody fragmentation experiments.

The Antibody Clean Up kit is designed for the rapid clean up of antibody solutions using a combination of our Pearl™ IgG Purification Resin to remove the protein stabilizers and SpinOUT™ desalting columns to ensure the antibody solutions are in an optimal buffer for clean up. The Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e. BSA and gelatin) and allows the IgG molecules to pass through in a physiological buffer.

For the purification of ten 0.5ml IgG samples with up to 1% BSA and gelatin.

FEATURES

- Remove BSA and Gelatin protein stabilizers
- SpinOUT™ columns to ensure optimal conditons for antibody clean up
- Pearl™ IgG Purification Resin for antibody clean up
- Suitable for 10 x 0.5ml IgG Samples

APPLICATIONS

- Remove BSA & Gelatin protein stabilizers that interfere with antibody labeling, fragmentation and isotyping experiments

Cat. No.	Description	Size
786-803	Pearl™ Antibody Clean Up	10 x 0.5ml samples

IgA PURIFICATION

Immobilized Jacalin

Jacalin, or *Artocarpus integrifolia* lectin, is a tetrameric two-chain lectin with a molecular weight of 66kDa. Jacalin is a α-D-galactose binding lectin purified from jack-fruit (*Artocarpus integrifolia*) seeds. Applications include isolating IgA from human serum and colostrums, isolating human plasma glycoproteins and histochemistry. Jacalin also binds IgD.

FEATURES

- Binding Capacity: 1-3mg human IgA/ml resin
- Loading: ≈4.5mg jacalin/ml of resin
- Support: 6% cross-linked agarose

APPLICATIONS

- Preparing Human IgA free of contaminating IgG

Cat. No.	Description	Size
786-167	Immobilized Jacalin	2ml resin

Jacalin, Lyophilized

Artocarpus integrifolia lectin

Jacalin, or *Artocarpus integrifolia* lectin, is also available as a lyophilized protein.

Cat. No.	Description	Size
786-473	Jacalin, lyophilized	10mg

THIOPHILIC ADSORPTION

Thiophilic Resin

For thiophilic adsorption of IgG, IgM, IgY and protein purification

Thiophilic adsorption or thiophilic chromatography is a routinely used technique for the low cost, simple purification of immunoglobulins. Thiophilic adsorption was first developed by Porath et al in 1984 and is a group specific, salt-dependent purification technique that has distinct affinity towards immunoglobulins and α_2-macroglobulins. The thiophilic adsorption works on the principle that some proteins in high salt are able to bind to an immobilized ligand that contains a sulfone group in proximity to a thioether group. The bound proteins are then eluted in decreasing salt concentrations.

The thiophilic resin binds immunoglobulins, including IgG, IgY and IgM, from serum, ascites or tissue culture supernatants and the purified immunoglobulins are then eluted in a near neutral aqueous buffer. The thiophilic resin has a high binding capacity (~20mg/ml human IgG/ml resin) and a broad specificity for various species' immunoglobulin molecules.

Thiophilic adsorption has been used to purify other proteins including horseradish peroxidase[2], glutathione peroxidase[3], lactate dehydrogenase[4] and allergens[5].

Supplied with protocols for IgG purification, IgM purification, IgY purification and general protein purification.

The Thiophilic Adsorption kit is supplied with the thiophilic resin and all the necessary buffers for the rapid purification of immunoglobulin G (IgG) antibodies.

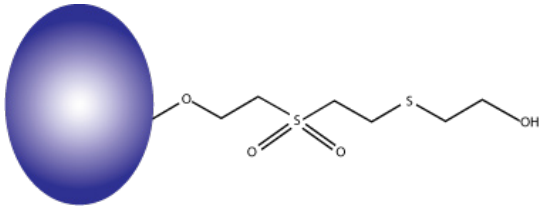


Figure 13: Structure of thiophilic group on agarose beads.

FEATURES

- Purify wide range of immunoglobulin molecules, including IgG, IgM and IgY
- High binding capacity (20mg human IgG/ml resin)
- Binds chicken immunoglobulin (IgY)
- Gentle elution conditions in very low salt and near neautral pH
- Adaptable to other proteins
- Enrichment alternative to ammonium sulfate precipitation

APPLICATIONS

- Purify immunoglobulins, including IgG, IgM and chicken IgY

REFERENCES

1. *Porath, J. et al (1984) In Physical Chemistry of Colloids and Macromolecules, Ed. Ranby, B. (Upsala, Sweden), p. 137*
2. *Chaga, G. et al (1992) Biomed. Chromatogr. 6:172*
3. *Huang, K. et al (1994) Biol. Trace Elem. Res. 46:91*
4. *Kminkova, M. & Kucera, J. (1998) Prep. Biochem. Biotechnol. 28:313*
5. *Goubran-Bostros, H. et al (1998) J. Chromatogr. B. Biomed. Sci. Appl. 710:57*

Cat. No.	Description	Size
786-266	Thiophilic Adsorption Kit	1 Kit
786-267	Thiophilic Resin	10ml resin
786-268	Thiophilic Resin	100ml resin

GLYCOPROTEIN PURIFICATION

FOCUS™ Glycoprotein

Glycoproteins are proteins that are post-translationally modified by the addition of carbohydrates. The carbohydrates are coupled to asparagine (N-linked) and serine/ threonine (O-linked) residues during passage through the endoplasmic reticulum and golgi apparatus. They are commonly found decorating the cell membrane with the carbohydrate moieties in the extracellular space. Glycosylated proteins play critical roles in cell signaling, inflammation, cell-to-cell adhesion and in the immune response.

FOCUS™ Glycoprotein rapidly fractionates glycoproteins that have terminal a-D-mannosyl and a-D glycosyl residues. FOCUS™ Glycoprotein utilizes spin columns containing the immobilized lectin Concanavalin A for rapid glycoprotein isolation.

FOCUS™ Glycoprotein kit was evaluated in the fractionation of Jurkat cells. After fractionation, fractions were analyzed by 1D electrophoresis stained with Reversible Zinc Stain™ and 2D electrophoresis stained with a fluorescent protein stain.

Shown below is the protein profile of Jurkat cells treated with FOCUS™ Glycoprotein. As expected the majority of the Jurkat cell proteome is removed in the flow through and washing steps as only a small percentage of the proteome is glycosylated. A large number of glycosylated proteins are isolated from the FOCUS™ Glycoprotein columns and the protein profiles change when each Glyco-Elution Buffer is used.

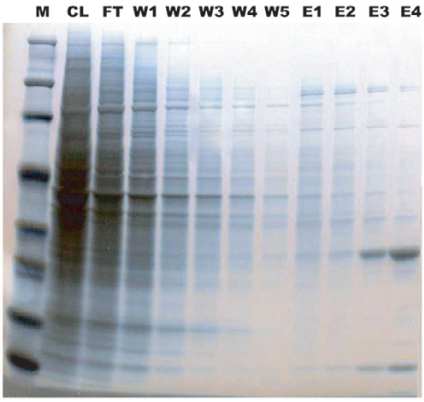


Figure 14: FOCUS™ Glycoprotein isolates multiple glycoproteins. Jurkat cells were lysed by sonication, centrifuged and the supernant (CL) loaded onto a FOCUS™ Glycoprotein column. The column ws centrifuged and the flow through (FT) collected. The column was washed (W1-5) and the glycoproteins were eluted with Glyco Elution Buffer I (E1-2), Glyco Elution Buffer II (E3) and then Glyco Elution Buffer III (E4). 10µl was loaded onto a SDS-PAGE gel, the proteins were resolved and visualized with Reversible Zinc Stain™.

In addition, the low abundant proteins are now easily visualized in the elution fractions as the majority of the proteome has been removed and due to the high sensitivity of the Reversible Zinc Stain™.

A comparison of the crude Jurkat cell lysate and an equal mix of the elutions E1, E2 and E3 are shown. Firstly, the use of the FOCUS™ Glycoprotein kit significantly reduces the complexity of the 2D map, making it easy to identify and isolate glycoprotein protein spots. Secondly, the concentration of the proteins is stronger due to the enrichment of the glycoproteins by the FOCUS™ Glycoprotein kit.

FOCUS™ Glycoprotein is ideal for the fractionation, enrichment and isolation of glycoproteins from a wide selection of samples, including tissues, cell lysates and serum. The fast and convenient spin column format bind and immobilize ~5mg glycoproteins and the enriched, eluted glycoproteins are ready for further analysis within 90 minutes.

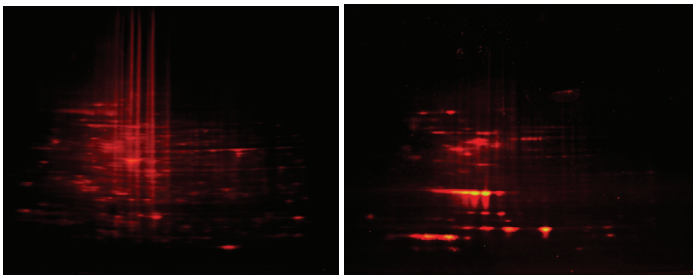


Figure 15: Comparison of crude Jurkat cell lysate (A) and glycoproteins (B) isolated with FOCUS™ Glycoprotein. Glycoproteins were isolated from Jurkat cells using FOCUS™ Glycoprotein kit as described in the Methods section. The first 3 elution fractions were combined and the eluents and a sample of the crude Jurkat cell lysate were treated with Perfect-FOCUS™ to prepare them for 2D electrophoresis. The first dimension was run on 11cm pH3-10 strips and the second dimension on 4-20% SDS polyacrylamide gels. Proteins were visualized with a fluorescent protein stain.

FEATURES

- Spin column protocol
- Uses a high capacity lectin binding resin (10-20mg/ml resin)
- Elution of glycoproteins within 90 minutes with a set of three rapid elution buffers

APPLICATIONS

- Fractionation and enrichment of glycoprotein
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-253	FOCUS™ Glycoprotein	10 Preps

Concanavalin A (Con A) Agarose

Concanavalin A (Con A) Agarose consists of Con A coupled to 4% agarose by the cyanogen bromide method. Con A is a tetrameric metalloprotein lectin isolated from Canavalia ensiformis (jack bean).

Con A is used for the purification of glycoproteins, polysaccharides and glycolipids as it binds molecules containing α-D-mannopyranosyl, α-D-glucopyranosyl and sterically related residues. Con A agarose has also be used in other application areas including purification of enzyme-antibody conjugates, purification of IgM and separation of membrane vesicles.

As stated above, Con A is a metalloprotein and to maintain its binding characteristics the presence of both Mn^{2+} and Ca^{2+} is essential. Each subunit of Con A utilizes one calcium and one manganese ion and these cations can be removed under acidic conditions abolishing the carbohydrate-binding activity.

FEATURES

- Binds α-D-mannopyranosyl, α-D-glucopyranosyl and sterically related residues
- Ligand Density: 10-16mg Con A/ml resin
- Capacity: 20-50mg thyroglobulin/ml resin
- Bead structure: 4% agarose

APPLICATIONS

- Purification/ enrichment of of glycoproteins, polysaccharides and glycolipids

Cat. No.	Description	Size
786-208	Concanavalin A (Con A) Agarose	10 x 0.75ml columns
786-216	Concanavalin A (Con A) Agarose	5ml resin
786-217	Concanavalin A (Con A) Agarose	10ml resin
786-218	Concanavalin A (Con A) Agarose	25ml resin

Carbohydrate Coupling Resin

Immobilize antibodies and other glycoproteins through carbohydrate groups.

For the covalent immobilization of carbohydrate containing molecules, including glycoproteins, to agarose beads.

Carbohydrate-containing molecules are treated with sodium meta-periodate to oxidize their cis-diol groups to aldehydes. The aldehydes spontaneously react with the hydrazide goups on the agarose beads to form stable covalent bonds. The stable nature allows the affinity resin to be used multiple times.

Ideal for the coupling of polyclonal antibodies as it allows for the optimal orientation of the antibodies for affinity purification.

The Carbohydrate Coupling kit includes 5 x 2ml Carbohydrate Coupling spin columns, SpinOUT™ desalting columns and sodium meta-periodate.

FEATURES

- Hydrazide activated agarose
- Capacity: 1-5mg antibody/ml resin
- Bead structure: 4% agarose

APPLICATIONS

- Purification/ enrichment of of glycoproteins, polysaccharides and glycolipids

Cat. No.	Description	Size
786-807	Carbohydrate Coupling Kit	For 5 columns
786-808	Carbohydrate Coupling Resin	5ml resin

LECTIN PURIFICATION

Immobilized D-Galactose

Purify lectins and galactose binding molecules

Designed for the rapid purification of lectins, galactosidases and other galactose-binding molecules. Ideal for the purification of agglutinins, lectins, toxins, glactose-binding, N-acetylgalactosamine-binding or carbohydrate binding molecules.

Immobilized D-Galactose consists of agarose covalently coupled to D-galactose.

FEATURES

- Ligand: Thio-α-D-galactose
- Binding Capacity: >20mg Jacalin/ml resin

APPLICATIONS

- Purification/ enrichment of of glycoproteins, polysaccharides and glycolipids

Cat. No.	Description	Size
786-391	Immobilized D-GalactoseKit	5ml resin

PHOSPHOPROTEIN PURIFICATION

FOCUS™ PhosphoRich™

FOCUS™ PhosphoRich™ is a ready-to-use kit that enriches phosphorylated proteins and phosphopeptides from complex biological samples. The kit contains spin columns that have a phosphoprotein binding resin with a binding capacity of ~20mg phosphorylated ovalbumin per column. The resin columns supplied with the kit can be reused, if regenerated and stored properly.

Figure 16: Various concentrations of phosphoprotein were loaded onto the FOCUS™ PhosphoRich™ columns and were washed extensively. The protein was rapidly eluted and the eluted proteins were resolved by SDS-PAGE. The phosvitin was visualized with the Reversible Zinc Stain™.

FEATURES

- Uses a phosphorylated protein binding spin column
- Rapid binding and elution of phosphoproteins

APPLICATIONS

- Enrichment of phosphorylated proteins and peptides
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry
- Suitable for proteomics and cell signaling studies

Cat. No.	Description	Size
786-255	FOCUS™ PhosphoRich™	5 Preps

ALBUMIN REMOVAL

AlbuminOUT™

Samples that contain a large abundance of albumin, such as plasma and cerebrospinal fluid, tend to mask identification and discovery of other less abundant proteins in2D gel electrophoresis and other studies. AlbuminOUT™ has been specifically developed for substantial removal of albumin from such samples.

The albumin removal method is based on binding of albumin with Cibachron™ Blue dye. AlbuminOUT™ has been optimized for removal of human albumin from samples. AlbuminOUT™ uses a rapid spin column method, where each column contains 0.2ml dye bound resins with capacity of >2mg human albumin per column. AlbuminOUT™ will remove >98% albumin from 5-50µl human plasma.

Spin column format allows removal of albumin within 10 minutes. High capacity blue-dye binding resin allows instantaneous binding and removal of albumin from human, pig, sheep, dog, rabbit, rat, and bovine samples. AlbuminOUT™ may also be used for removal of albumin from other species. Suitable for processing 25 or 50 samples.

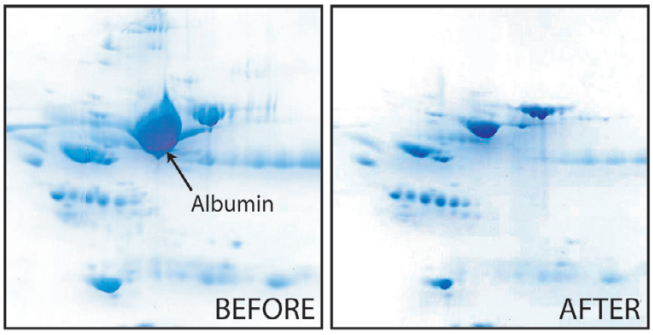


Figure 17: 2D analysis of whole human serum before (left) and after (right) treatment with AlbminOUT™.

FEATURES

- Removal of albumin from samples in less than 10 minutes
- Based on binding of albumin with Cibachron™ Blue dye
- Column capacity >2mg human albumin per column
- Removes >98% albumin from 5-50µl human plasma

APPLICATIONS

- Removal of albumin from biological samples such as plasma and cerebrospinal fluid

Cat. No.	Description	Size
786-251	AlbuminOUT™	25 preps
786-252	AlbuminOUT™	50 preps

DESALTING & BUFFER EXCHANGE

Spin-OUT™

For desalting and buffer exchange

The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein and nucleic acid solutions ranging from 5µl through to 4ml sample volumes. The SpinOUT™ columns are available in two MWCO sizes. Simply apply the sample and then centrifuge to recover protein/nucleic acids with the column retaining >95% of the salts and small molecules (<1,000Da).

Spin-OUT™ GT-600 is for the purification of proteins >6kDa and nucleic acids larger than 10bp.

Spin-OUT™ GT-1200 is for the purification of proteins >30kDa and the removal of molecules >1,500Da.

FEATURES

- 5 sizes available for sample volumes of 5µl to 4ml
- Spin format for rapid purification

CITED REFERENCES

Taggert, C. et al (2005) J. Exp. Med. 202: 1659
Tripodi, K et al (2005) Plant Physiol. 139: 969

Cat. No.	Description	Size	Resin Bed (ml)	Sample Load (ml)
786-703	SpinOUT™ GT-600, 0.1ml	25 columns	0.1	0.005-0.02
786-170	SpinOUT™ GT-600, 1ml	10 columns	1	0.05-0.1
786-171	SpinOUT™ GT-600, 3ml	10 columns	3	0.1-0.5
786-704	SpinOUT™ GT-600, 5ml	5 columns	5	0.5-2
786-705	SpinOUT™ GT-600, 10ml	5 columns	10	0.5-4
786-706	SpinOUT™ GT-1200, 0.1ml	25 columns	0.1	0.005-0.02
786-172	SpinOUT™ GT-1200, 1ml	10 columns	1	0.05-0.1
786-173	SpinOUT™ GT-1200, 3ml	10 columns	3	0.1-0.5
786-707	SpinOUT™ GT-1200, 5ml	5 columns	5	0.5-2
786-708	SpinOUT™ GT-1200, 10ml	5 columns	10	0.5-4

SpinOUT™ for PCR

SpinOUT™ PCR is for the cleaning of PCR products. PCR-20 removes contaminating products from PCR products, including <20bp primers, dNTPs and salts. PCR-32 removes PCR products from <32bp primers, dNTPs and salts. For more information see the DNA Clean Up & Concentration section.

Cat. No.	Description	Size
786-174	SpinOUT™ PCR-20	10 columns
786-175	SpinOUT™ PCR-32	10 columns

DETERGENT REMOVAL

G-Biosciences offers a range of detergent removal systems that use either a rapid column based system or a precipitation system.

Our products are designed to remove a wide variety of detergents, including SDS, Tween® 20, Triton® X-100, Triton® X-114, Nonidet® P-40, CTAB, CHAPS, deoxycholate and Lubrol®.

DetergentOUT™ GBS10

Detergents are essential for protein solubility during protein extraction and sample preparation, especially when working with hydrophobic proteins. The presence of high concentrations of detergents in protein samples can impair ELISA, IEF, protease digestion of proteins and suppress peptide ionization when analyzed by mass spectrometry.

DetergentOUT™ GBS10 resin removes free, unbound anionic, nonionic or zwitterionic detergents (e.g. SDS, Triton® X-100 or CHAPS) from aqueous protein and peptide samples with minimal sample loss.

The DetergentOUT™ GBS10 columns were shown in independent studies to be fully compatible with DI-QTOF and LC-MS/MS. The use of the DetergentOUT™ GBS10 columns significantly increased the number of peptide spectra detected. In addition, the DetergentOUT™ GBS10 columns have a high binding capacity for detergents, i.e. 6mg SDS and 14mg Triton® X-100 by every ml settled resin.

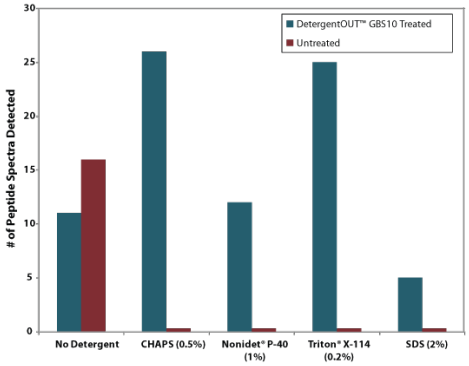


Figure 18: DetergentOUT™ GBS10 removes detergent & allows detection of peptide fragments by mass spectrometry. 500µg phosphorylase B was digested in solution & the indicated amount of detergent was added. Samples were treated with DetergentOUT™ GBS10. Number of peptide spectra were determined as per the protocol of Alvarez, S. et al.

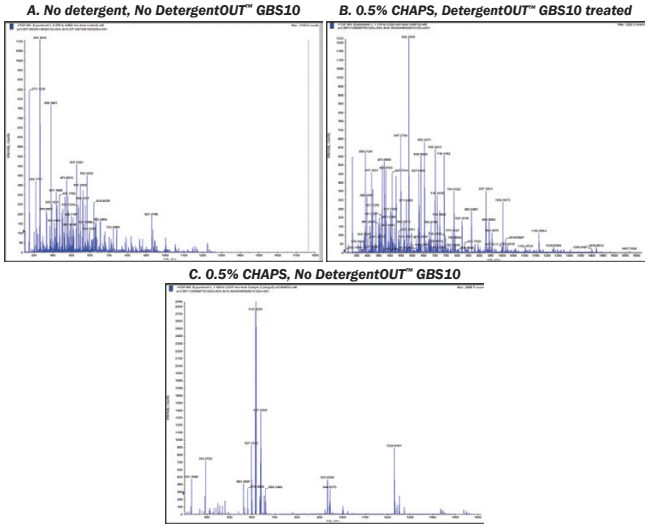


Figure 19: DetergentOUT™ GBS10 enhances mass spectrometry spectra. 5µg/µl protein mixture (BSA, cyctochrome C & phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B & C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 & compared to an untreated sample (Panel C). Spectra were generated per Alvarez et al.

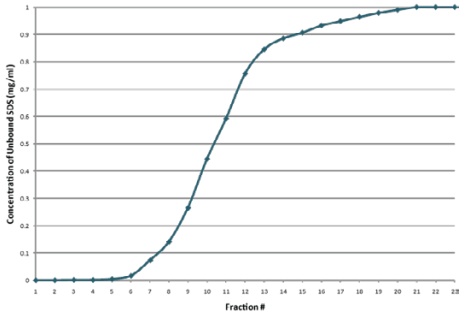


Figure 20: DetergentOUT™ GBS10 retains ≤6mg SDS per ml settled resin. SDS solution was continuously applied to DetergentOUT™ GBS10 column. The graph depicts the amount of SDS detected in the flow-through. SDS was not detected until fraction 7, so after 12mg SDS had been retained by the 2ml of DetergentOUT™ GB-S10 resin, resulting in a 6mg/ml settled resin binding capacity.

Detergent	% Removed	BSA	Phosphorylase B	Cytochrome C	E.coli Lysate
Triton X-100, 2%	>99	>90	>91	>92	>93
Triton X-114, 2%	>96	>99	>98	>97	>91
Nonidet P-40, 1%	>96	>93	>95	>91	>91
Brij 35, 1%	>99	>98	>99	>97	>91
SDS, 2.5%	>99	>96	>97	>92	>90
Sodium deoxycholate, 5%	>99	>99	>99	>98	>95
CHAPS, 3%	>99	>92	>95	>92	>91
Octyl glucoside, 5%	>99	>93	>95	>96	>91
Lauryl maltoside, 1%	>97	>99	>99	>99	>91

Table 4: Comparison of detergent removal rates & percentage of protein recovery with DetergentOUT™ GBS10

FEATURES

- Easy-to-use, spin-format columns
- Rapid removal of free detergents
- Minimal sample loss
- Suitable for anionic, non-ionic & zwitterionic detergents
- Available for sample volumes ranging from 10µl to 1,250µl

APPLICATIONS

- Detergent removal from protein & peptide solutions
- Detection of peptide fragments by Mass spectrometry
- Enhances Mass spectrometry Spectra
- Ideal for downstream analysis by mass spectrometry & other techniques

CITED REFERENCES

Alvarez, S. et al (2010) Poster presented as part of the 58th ASMS Conference on Mass Spectrometry and Allied Topics, May 23-27, 2010, Salt Lake City, Utah
Sivakumar, S. et al (2007) J. Biol. Chem. 282: 7312
Urdaneta, S. et al (2006) J. Human Lact. 22: 61
Higgins, D. et al (2005) Anitmicrob. Agents Chemother. 49: 1127
Fisher, J. and Margulies, S. (2002) Am. J. Physiol. Lung Cell Mol. Physiol. 283: L737
Baizman, E. et al (2000) Microbiology 146: 3129

Cat. No.	Description	Sample Size (µl)	Resin (µl)	Size
786-154	DetergentOUT™ GBS10-125	10-30	125	10 columns
786-155	DetergentOUT™ GBS10-800	30-200	800	10 columns
786-156	DetergentOUT™ GBS10-3000	200-750	3,000	10 columns
786-157	DetergentOUT™ GBS10-5000	500-1,250	5,000	10 columns
786-159	DetergentOUT™ GBS10 Resin	-	-	10ml resin

DetergentOUT™ Tween®

Removal of Tween® (polysorbate) detergents

A spin column format detergent removal resin for polysorbate or Tween® detergents or surfactants. DetergentOUT™ Tween® specifically removes polysorbate detergents without significant loss of proteins, dilution of the protein solution, or change to the buffer composition of the protein solution.

For other detergents, we highly recommend our DetergentOUT™ GBS10 columns and resin. The DetergentOUT™ GBS10 shows greater efficiency of detergent removal and protein recovery for other detergents, including SDS, CHAPS, Triton®, Nonidet® and Brij®

Cat. No.	Description	Size
786-214	Detergent*OUT*™ Tween®, Micro	10 columns
786-215	Detergent*OUT*™ Tween®, Medi	10 columns

OrgoSol DetergentOUT™

Suitable for hydrophobic proteins, removes detergents and concentrates protein solutions

OrgoSol DetergentOUT™ is suitable for removal of detergents from protein solutions, including hydrophobic protein solutions and is compatible with all detergent types. Its performance is not dependent on the concentration of detergents in the solution, is highly flexible and can be used to process small and large sample volumes.

OrgoSol DetergentOUT™ first concentrates the protein solution through precipitation and then the detergent is extracted and removed with the supplied OrgoSol™ buffer. The proprietary precipitation agent ensures >99% protein recovery, however precipitation may result in some loss of a protein's biological activity.

Two sizes are offered: Micro Kit for processing up to a total of 10ml protein solution and Medi Kit for processing up to a total of 30ml protein solution, either in a single or multiple experiments.

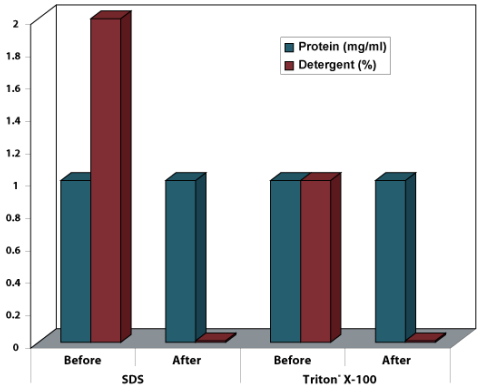


Figure 21: Removal of Detergent. Hydrophobic nuclear fraction proteins (1mg/ml) in 2% SDS and 1% Triton® X-100 before and after OrgoSol DetergentOUT™ treatment.

CITED REFERENCES

Troese, M.J. et al (2011) Infect. Immun. 79: 4696

Cat. No.	Description	Size
786-127	OrgoSol Detergent*OUT*™, Micro	For 10ml
786-128	OrgoSol Detergent*OUT*™, Medi	For 30ml

ENDOTOXIN REMOVAL

EndotoxinOUT™

Rapid removal of endotoxins & pyrogens

For the rapid removal of endotoxins/pyrogens (LPS, lipopolysaccharides) from samples.

EndotoxinOUT™ consists of 6% cross-linked agarose covalently linked to polymyxin B to bind and remove harmful pyrogens from a solution. Polymyxin B is a family, polymyxin B1 and B2, of antibiotics that bind to the negatively charged site of the lipid A portion of bacterial lipopolysaccharide layer neutralizing the endotoxic activity.

The covalent coupled agarose and polymyxin B is a stable matrix that resists leaching. An ideal product for the clean up of buffers, cell culture media, protein solutions, nucleic acid (DNA) samples and pharmacological components.

FEATURES

- Polymyxin B Sulfate immobilized on 6% cross-linked agarose
- Capacity: ≥9995 endotoxin units (EU) removed by 1ml resin from 5ml test containing 10,000EU
- ≥99.95% removal
- Reusable at least 10 times

APPLICATIONS

- Clean up of buffers, cell culture media, protein solutions and pharmacological components
- Removal of endotoxins from nucleic acid (DNA) samples

Cat. No.	Description	Size
786-367	EndotoxinOUT™	10ml resin
786-368	EndotoxinOUT™	1L resin
786-369	EndotoxinOUT™	5 x 1ml columns

DISPOSABLE COLUMNS

Spin Column, <0.1ml

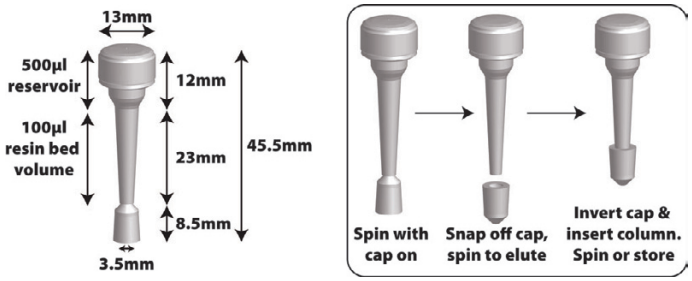


Figure 22: Spin Column, <0.1ml.

Unique design with the snap off end converting to a closure for the column for easy manipulation and use.

FEATURES

- Column volume: 600µl
- Resin volume: 5-100µl
- Filter type: Polyethylene filter, ~30µm pore size
- Fits in 1.5 and 2ml centrifuge tubes

Cat. No.	Description	Size
786-718	Spin Column, <0.1ml	25
786-719	Spin Column, <0.1ml	50

Spin Column, 1ml

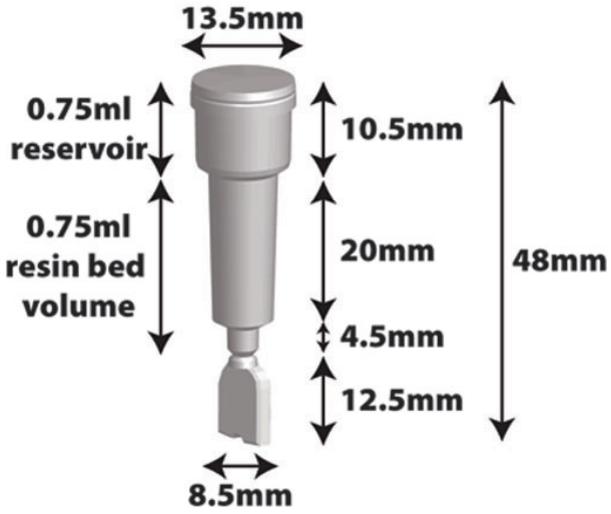


Figure 23: Spin Column, 1ml.

FEATURES

- Column volume: 1.5ml
- Resin volume: 750µl
- Filter type: Polyethylene filter, ~20µm pore size
- Cap and rubber stoppers included
- Fits in 1.5 and 2ml centrifuge tubes

Cat. No.	Description	Size
786-198	Spin Column, 1ml	10
786-720	Spin Column, 1ml	25
786-721	Spin Column, 1ml	50

Spin Column, 2ml

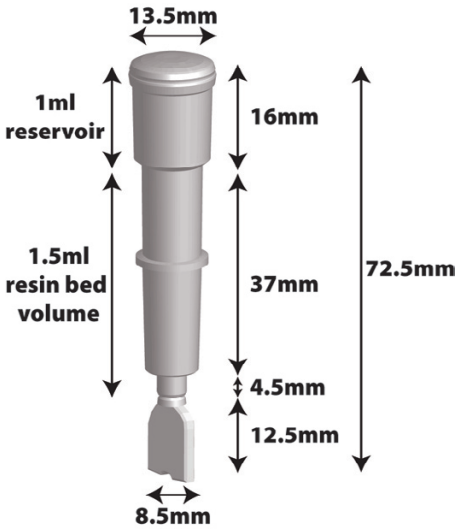


Figure 24: Spin Column, 2ml.

FEATURES

- Column volume: 2.5ml
- Resin volume: 1.5ml
- Filter type: Polyethylene filter, ~20µm pore size
- Cap and rubber stoppers included
- Fits 15ml conical centrifuge tubes

Cat. No.	Description	Size
786-722	Spin Column, 2ml	25
786-723	Spin Column, 2ml	50

Spin Column, 3ml

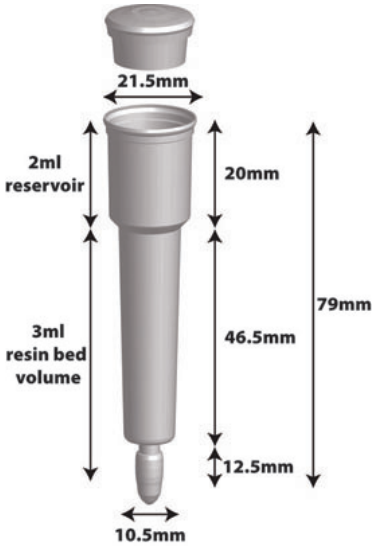


Figure 25: Spin Column, 3ml.

FEATURES

- Column volume: 5ml
- Resin volume: 3ml
- Filter type: Polyethylene filter, ~30µm pore size
- Cap and rubber stoppers included
- Fits 15ml conical centrifuge tubes

Cat. No.	Description	Size
786-724	Spin Column, 3ml	25
786-725	Spin Column, 3ml	50

Spin Column, 5ml

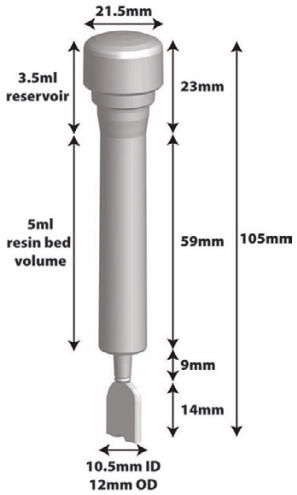


Figure 26: Spin Column, 5ml.

FEATURES

- Total volume: 8ml
- Resin volume: 5ml
- Filter type, pore size: Polyethylene filter, ~30µm pore size
- Fits 15ml conical centrifuge tubes
- Cap and rubber stoppers included

Cat. No.	Description	Size
786-726	Spin Column, 5ml	10

Spin Column, 10ml

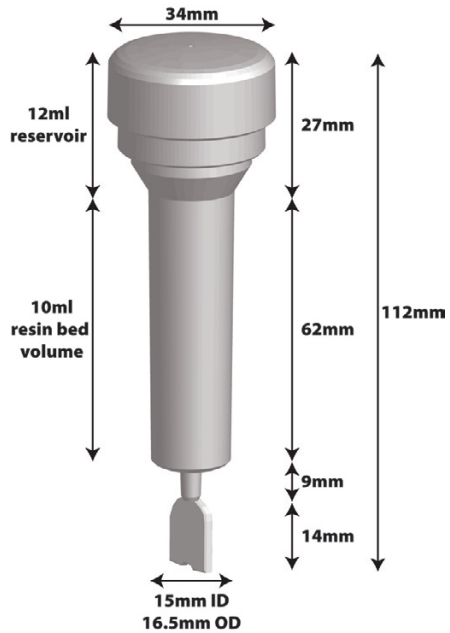


Figure 27: Spin Column, 10ml.

FEATURES

- Total volume: 22ml
- Resin volume: 10ml
- Filter type, pore size: Polyethylene filter, ~30µm pore size
- Fits 50ml conical centrifuge tubes
- Cap and rubber stoppers included

Cat. No.	Description	Size
786-727	Spin Column, 10ml	10

Gravity Flow Column, 5ml

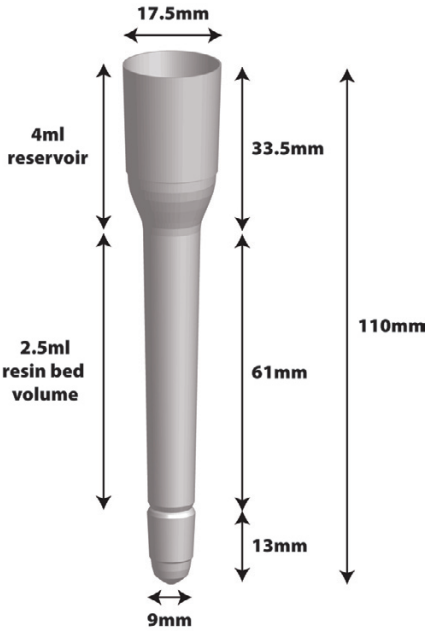


Figure 28: Gravity Flow Column, 5ml.

The 5ml Columns have an internal volume of 6.5ml and is designed for small scale gravity flow purifications.

FEATURES

- Total Volume: 6.5ml
- Resin Volume: 2.5ml
- Reservoir Volume: 4ml
- Closure: Plastic Stopper
- Cap: Push in cap
- Frit: 1.5mm ~30µm hydrophobic polyethylene

Cat. No.	Description	Size
786-169	Column, 5ml	25

Gravity Flow Column, 20ml

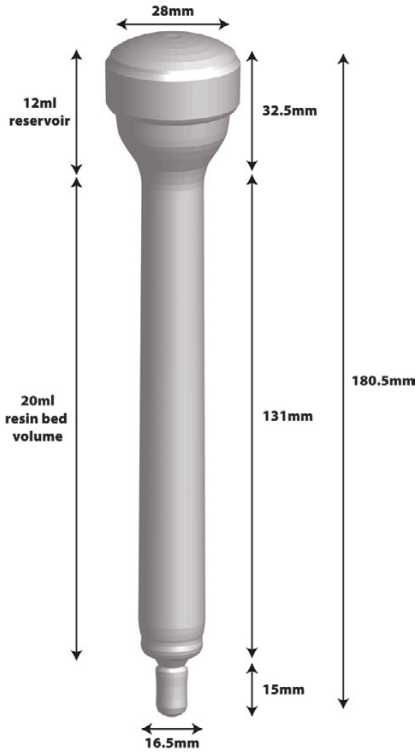


Figure 29: Gravity Flow Column, 20ml.

The 20ml Columns have an internal volume of 32ml and is designed for small scale gravity flow purifications. The resin bed volume is 20ml. Supplied with screw caps and stoppers.

FEATURES

- Total Volume: 32ml
- Resin Volume: 20ml
- Reservoir Volume: 12ml
- Graduated
- Closure: Plastic Stopper
- Cap: Screw cap
- Frit: 3mm ~30µm hydrophobic polyethylene

Cat. No.	Description	Size
786-197	Column, 20ml	25

G-Biosciences Product Line Overview

Estimation	7 Assays	CB-X Non Interfering SPN RED 660 dotMETRIC BCA CB	
Isolation	Extraction & Lysis	Sample Grinding Lysis Buffers	Mild Denaturing Strong Chaotropic Specialized
	Fractionation & Enrichment	12 Fractionation Kits Dialysis (Micro) Concentration	
	Sample Preparation	Contamination Removal	Desalting Detergent Removal
	Reagents	Protease Inhibitors	General Cocktails Species Specific Individual 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
		GST Tag Biotin Tag CBP Tag	Glutathione Resin Streptavidin Resin Calmodulin Resin
	Activated Resins	Sulfhydryl reactive Amine reactive Carboxyl reactive	
	Antibody Purification	Drug/ Steroid reactive Protein A or G Pearl Resin	
Modification	Labeling	Biotin Fluorescent Dye Enzyme (HRP/AP)	
	Crosslinkers Reducing Agents Alkylating Agents Protein Cleavage Iodination Amino Acid Side Chain Modifiers		
Antibody	Production	Carrier Proteins	BSA KLH HyperCarrier
	Purification	Peptide Coupling Protein A or G Resin Activated Resins Pearl Resin Thiophilic Resin	
	Fragmentation	Ficin Pepsin Papain	
SAM Methyltransferase	Continuous, Enzymatic Assays Lactate Dehydrogenase (LDH)		
Cell Toxicity & Proliferation	SRB WST-1		
Apoptosis	Caspase	Assays Substrates Inhibitors	
Protease	Inducers Assays Inhibitors		
Phosphatase Peroxide			
B-Galactosidase	CPRG Fluorescent (MUG)		
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 Isolation		

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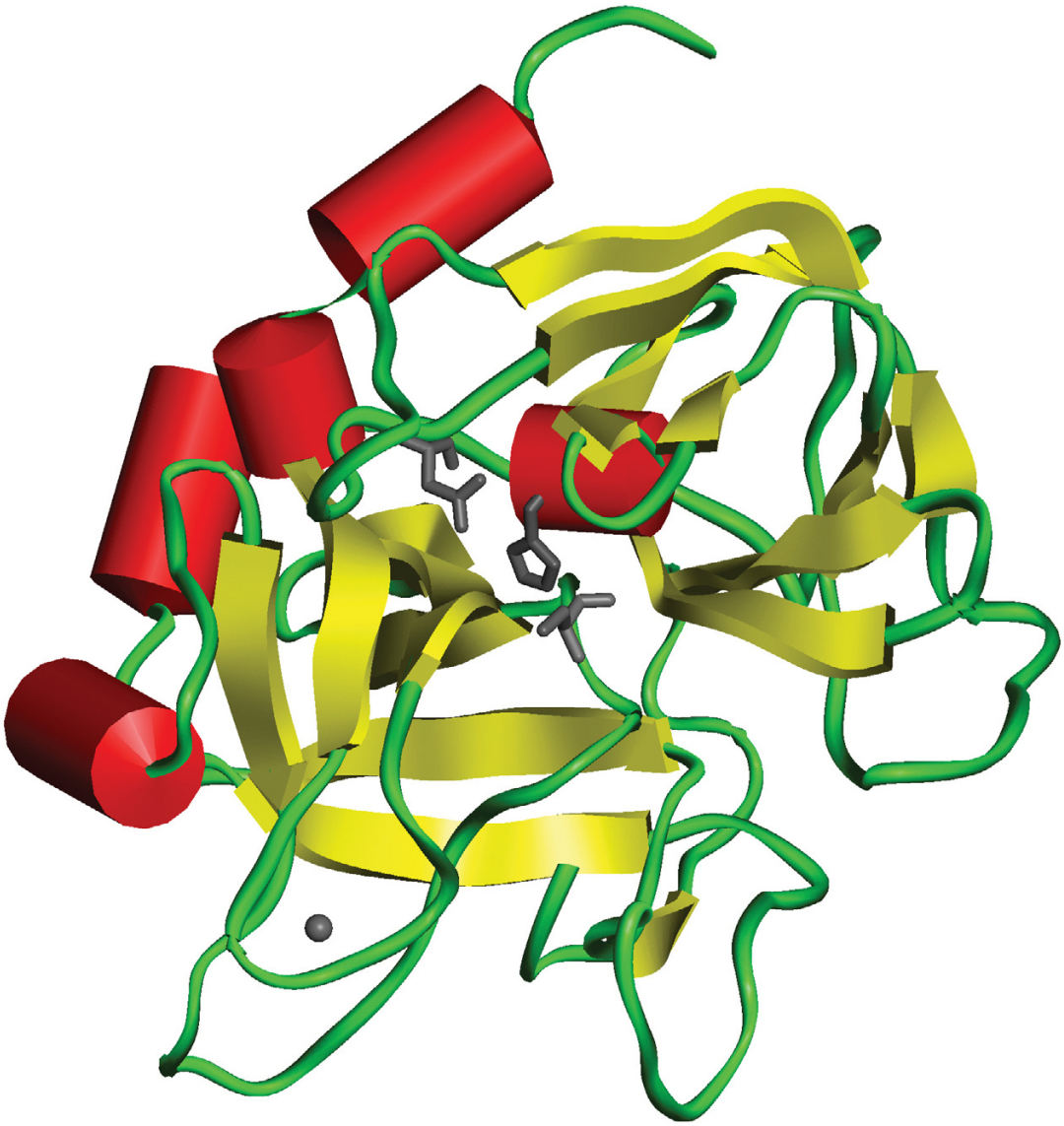

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