1053_88391B 12/9/05 2:36 PM Page 1

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Enchant™ IgG Purification and Depletion Kits

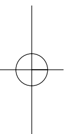
- Simple, easy to use Protein A or Protein G affinity columns for IgG purification and depletion

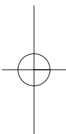
Ordering Information

Prod. No.	Description	Pkg
5300-IGGPROA	Enchant Protein A IgG Purification/Depletion Kit	50 purifications
5300-IGGPROG	Enchant Protein G IgG Purification/Depletion Kit	10 purifications

1053_88391B 12/9/05 2:36 PM Page 2

Life Sciences





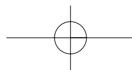


Table of Contents

Section	Page
Introduction	2
Specifications	3
Frequently Asked Questions	5
Protocols	9
Protein A	9
Protein G	12
Sample Desalting	15
Troubleshooting	18
Complementary Products	19
Warning	19

Note: *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact our Technical Services Department*

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Introduction

The Enchant IgG Purification and Depletion kits offer a flexible platform to both purify and deplete IgG from serum, plasma, ascites fluid and tissue culture supernatants. The re-usable columns utilize either Protein A or Protein G affinity resin to purify/deplete samples from a wide variety of species. The kits include all of the reagents necessary to complete the assay including; affinity columns, desalting columns and the required binding and Elution Buffers.

Affinity purification via Protein A and G are two of the most widely recognized and simplest methods for antibody purification and depletion. Protein A and Protein G are bacterial cell wall proteins that have specificity to the Fc portion of certain immunoglobulins. Protein A, which contains four IgG binding regions, is a 42,000 Dalton single chain polypeptide isolated from the cell wall of the bacteria *Staphylcoccus aureus*. Derived from the bacterial walls of Group G Streptococci, Protein G is a 32,000 Dalton polypeptide with two binding sites for the F_c region of IgG. Both proteins have been widely characterized for their affinity to various types of immunoglobulins. Although both Protein A and Protein G have a strong affinity for IgG antibodies, they do have varying affinities to other immunoglobulin subclasses and isotypes.

Specifications

Binding Capacity

Protein A Columns: 11 - 19 mg of human IgG/mL of gel and
6 - 8 mg of mouse IgG/mL of gel
Protein G Columns: 10 - 15 mg of human IgG/mL of gel

Storage Conditions

Store at 4 °C upon receipt. Affinity and desalting columns should be kept upright. Shipping occurs at ambient temperature.

Shelf Life

One year from the date of purchase, if stored properly.

Enchant™ IgG Purification Kit Components

Protein A Kit

Protein A Affinity Purification Columns
Quantity: 5 columns
Solid Support: Protein A covalently crosslinked to 6% beaded agarose
Column Volume: 1 mL
Columns are supplied in an aqueous slurry containing 0.02% sodium azide (bacteriocide).

Protein A IgG Binding Buffer
Quantity: 1 Liter
pH: 8.0
Note: Contains EDTA as preservative

Protein A IgG Elution Buffer
Quantity: 500 mL
pH: 2.8

Gel Filtration Desalting Columns
Quantity: 5 columns
Solid Support: Porous cellulose beads
Particle Size: 40 - 100 µm
Column Volume: 5 mL
Void Volume: 1.75 mL
Molecular Weight Cut Off: 5,000 Daltons

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Specifications (cont.)

Enchant™ IgG Purification Kit Components
Protein G Kits
Protein G Affinity Purification Column
Quantity: 1 column
Solid Support: Protein G covalently crosslinked to 6% beaded agarose
Column Volume: 2 mL
Columns are supplied in an aqueous slurry containing 0.02% sodium azide (bacteriocide)

Protein G IgG Binding Buffer
Quantity: 240 mL
pH: 5.0
Note: Contains 0.02% sodium azide (bacteriocide)

Protein G IgG Elution Buffer
Quantity: 120 mL
pH: 2.8

Gel Filtration Desalting Columns
Quantity: 5
Solid Support: Porous cellulose beads
Particle Size: 40 - 100 µm
Column Volume: 5 mL
Void Volume: 1.75 mL
Molecular Weight Cut Off: 5,000 Daltons

Frequently Asked Questions

Q: How do I select which affinity column will be optimal for purifying or depleting my antibody of interest?

A: There are differences in the affinity, which Protein A and Protein G have for antibodies from different species and for the different isotypes within a species. The following chart will provide a guideline for selecting the proper affinity column based on the antibody class and isotype being purified.

Species	Antibody Class	Protein A	Protein G
Human	**Total IgG**	+++	+++
Human	IgG_1	+++	+++
Human	IgG_2	+++	+++
Human	IgG_3	+	+++
Human	IgG_4	+++	+++
Human	IgM	+	None
Human	IgD	None	None
Human	IgE	++	None
Human	IgA	+	None
Human	IgA_1	+	None
Human	IgA_2	+	None
Human	Fab	+	+
Human	ScFv	+	None

Key:

Strong Binding = +++
Medium Binding = ++
Weak Binding = +
No Binding = None

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Frequently Asked Questions (cont.)

Species	Antibody Class	Protein A	Protein G
Mouse	**Total IgG**	**+++**	**+++**
Mouse	IgG_1	+	++
Mouse	IgG_{2a}	+++	+++
Mouse	IgG_{2b}	+++	+++
Mouse	IgG_3	+++	+++
Mouse	IgM	None	None
Rat	**Total IgG**	**+**	**++**
Rat	IgG_1	+	++
Rat	IgG_{2a}	None	+++
Rat	IgG_{2b}	None	+
Rat	IgG_{2c}	+++	+++
Horse	**Total IgG**	**+**	**+++**
Horse	$IgG_{(ab)}$	+	None
Horse	$IgG_{(c)}$	+	None
Horse	$IgG_{(T)}$	None	+++
Cow	**Total IgG**	**+**	**+++**
Cow	IgG_1	+	+++
Cow	IgG_2	+++	+++

Key:

Strong Binding = +++
Medium Binding = ++
Weak Binding = +
No Binding = None

Frequently Asked Questions (cont.)

Species	Antibody Class	Protein A	Protein G
Goat	**Total IgG**	**+**	**+++**
Goat	IgG_1	+	+++
Goat	IgG_2	+++	+++
Sheep	**Total IgG**	**+**	**+++**
Sheep	IgG_1	+	+++
Sheep	IgG_2	+++	+++
Cat	**Total IgG**	**+++**	**+**
Chicken	**Total IgY**	**None**	**None**
Dog	**Total IgG**	**+++**	**+**
Donkey	**Total IgG**	**++**	**+++**
Guinea Pig	**Total IgG**	**+++**	**+**
Hamster	**Total IgG**	**++**	**++**
Pig	**Total IgG**	**+++**	**+**
Rabbit	**Total IgG**	**+++**	**+++**
Rhesus Monkey	**Total IgG**	**+++**	**+++**

Key:

Strong Binding = +++
Medium Binding = ++
Weak Binding = +
No Binding = None

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Frequently Asked Questions (cont.)

Q: Can I use the Protein A and G columns to deplete IgG from my serum or plasma samples?

A: Yes. The Protein A and G columns can be used to either purify or deplete IgG from your sample. See Protocol section for detailed protcols.

Q: Can I neutralize my Protein A purified fractions?

A: Yes. To neutralize the purified 1 mL fractions, add either 100 µL of Binding Buffer or 50 µL of 1M Tris, pH 9.5. The advantage of using the 1M Tris solution will allow you to keep your samples in smaller volumes.

Q: Can I neutralize my Protein G purified fractions?

A: Yes. Prepare a high ionic strength alkaline neutralization buffer. 1M Phosphate, pH 7.5 - 9.0 or 1M Tris, pH 7.5 - 9.0 are sufficient. If using a neutralization buffer with a pH of 7.5, add 100 µL to each 1 mL fraction. If using a neutralization buffer with a pH of 9.0, add 50 µL to each 1 mL fraction.

Q: What is the gravity flow rate of the desalting columns?

A: The flow rate of the desalting columns is approximately 100 µL per minute. Flow on the desalting column will stop if the sample drains down to the disc on the top of the column bed.
Note: You should not allow the gel bed in the column to dry out.

Q: What is the binding capacity of the Protein A and Protein G affinity columns?

A: The total IgG content of serum is typically 10 - 15 mg/mL, while the specific IgG of interest only accounts for 2 - 5% of this total. Therefore, the binding capacity of the affinity columns is enough to purify 1 - 2 mL of sample.

Q: Can I reuse my Protein A and Protein G affinity purification columns?

A: Yes. The Protein A and Protein G affinity purification columns can be washed and reused. Each column can be used for 10 purifications without loss of binding. See Protocol section for column regeneration and storage procedures.

Protocols

Enchant™ IgG Purification Kit with Protein A

It is important to note that when purifying antibodies, they may not exhibit the expected binding affinity for Protein A. We recommend a test experiment to ensure the binding affinity for your specific antibody.

Protein A Affinity Column Equilibration

1. Bring all kit components and sample to same temperature. Procedure can be done either at room temperature or in a cold room. If all components are not at the same temperature, air bubbles in the column could result.
2. Open the Protein A affinity column by removing the top cap first. Decant the storage buffer. Remove the cap on the bottom of the column. Place Protein A affinity column in a standard test tube or test tube holder.

   Note: Retain caps for storage and affinity column regeneration.
3. Pipette 5 mL of Protein A Binding Buffer to the Protein A affinity column. Allow the Binding Buffer to pass through the column. Collect Binding Buffer in a standard test tube. This will equilibrate the Protein A affinity column.

IgG Depletion with Protein A

1. Dilute 1 mL of sample 1:1 with Protein A Binding Buffer before applying to Protein A affinity column.

   Note: Plasma samples will become cloudy/opaque after dilution with the Protein A Binding Buffer. This is a result of lipoprotein precipitation. If this occurs, centrifuge the sample at 10,000 x g for 15 minutes (or until clear). Apply cleared supernatant to the Protein A affinity column.
2. Apply diluted sample to Protein A affinity column and allow sample to pass through the column.
3. Collect 1 mL fractions and set aside for analysis.
4. Add 10 - 15 mL of Protein A Binding Buffer to the column to wash IgG depleted sample through.
5. Continue to collect 1 mL fractions to collect remainder of flow through.
6. Measure the absorbance of each fraction at the 280 nm reading. The fractions with the highest absorbance ratings will contain you IgG depleted sampled. This will typically be fractions 2 - 4.

1053_88391B 12/9/05 2:36 PM Page 12

Protocols (cont.)

IgG Purification

1. Dilute sample (serum, plasma, ascites fluid, or tissue culture supernatant) 1:1 with Binding Buffer before applying to the Protein A affinity column.

   Note: Plasma samples may become cloudy/opaque after dilution with the Protein A Binding Buffer. This is a result of lipoprotein precipitation. If this occurs, centrifuge the sample at 10,000 x g for 15 minutes (or until clear).
2. Transfer Protein A affinity column to second test tube and add the diluted sample to the Protein A affinity column.
3. Allow sample to flow through the Protein A affinity column.
4. Collect the filtrate solution in a test tube and set aside for analysis of binding efficiency.
5. Pipette 15 mL of Protein A Binding Buffer to wash the Protein A affinity column.
6. To verify that all unbound proteins are washed away, collect filtrate in 2 mL fractions. Measure their absorbance readings at 280 nm. The last fractions should have absorbance readings that are the same as the Binding Buffer.

IgG Elution

1. Pipette 5 mL of Protein A Elution Buffer to the Protein A affinity column to recover the bound IgG.
2. Collect the filtrate in numbered collection tubes. For collection purposes, it is important to know where your sample is in the column. The gel bed holds approximately 1 mL of solution. If you have a 1 mL of sample, then the entire sample will fit into the gel bed. As the sample passes through the column, the first milliliter that comes through the column will contain some of the Elution Buffer and some of the purified IgG. The next 2 - 3 fractions will contain the majority of the purified IgG.

   Note: We recommend that you collect the sample in 0.5 mL to 1.0 mL fractions.

Protocols (cont.)

Sample Neutralization

1. To neutralize the purified 1 mL fractions, add either 100 µL of Protein A Binding Buffer or 50 µL of 1M Tris, pH 9.5 to each 1 mL fraction. The advantage of using the 1M Tris solution will allow you to keep your samples in smaller volumes.

Purified IgG Identification

1. Blank a spectrophotometer set at 280 nm. You can use deionized water or 1 mL of Protein A Elution Buffer with 50 - 100 µl of neutralization buffer to blank the spectrophotometer.
2. Measure the absorbance of each fraction at the 280 nm reading.
3. The fractions with the highest absorbance readings will contain your purified IgG.
4. Pool only the purified fractions with the highest absorbance readings.

   Note: You can read the absorbance of the fractions before or after neutralization.

Protein A Affinity Column Regeneration

1. To regenerate the column, wash the Protein A affinity column with 8 mL of 0.1M Citric Acid, pH 3.0 (adjust pH with NaOH). To store the Protein A affinity column, wash it with 5.0 mL of 0.02% sodium azide containing water. Recap the column when 1 - 2 mL of water remains above the disc at the top of the column. Recap column by replacing the bottom cap first. Store column upright at 4 °C.

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Protocols (cont.)

Enchant™ IgG Purification Kit with Protein G

It is important to note that when purifying antibodies, they may not exhibit the expected binding affinity for Protein G. We recommend a test experiment to ensure the binding affinity for your specific antibody.

Protein G Affinity Column Equilibration

1. Bring all kit components and samples to same temperature. Procedure can be done either at room temperature or in a cold room. If all components are not at the same temperature, air bubbles in the column could result.
2. Open the Protein G affinity column by removing the top cap first. Discard the storage buffer. Remove the cap on the bottom of the column. Place Protein G affinity column in a standard test tube or test tube holder.

   Note: Retain caps for storage and affinity column regeneration.
3. Pipette 5 mL of Protein G Binding Buffer to the Protein G affinity column. Allow the Binding Buffer to pass through the column. Collect Binding Buffer in a standard test tube. This will equilibrate the Protein G affinity column.

IgG Depletion with Protein G

1. Dilute 2 mL of sample 1:1 with Protein G Binding Buffer before applying to Protein G affinity column.

   Note: Plasma samples will become cloudy/opaque after dilution with the Protein G Binding Buffer. This is a result of lipoprotein precipitation. If this occurs, centrifuge the sample at 10,000 x g for 15 minutes (or until clear). Apply cleared supernatant to the Protein G affinity column.
2. Apply diluted sample to Protein G affinity column and allow sample to pass through the column.
3. Collect 1 mL fractions and set aside for analysis.
4. Add 10 - 15 mL of Protein G Binding Buffer to the column to wash IgG depleted sample through.
5. Continue to collect 1 mL fractions to collect remainder of flow through. Measure the absorbance of each fraction at the 280 nm reading. The fractions with the highest absorbance ratings will contain you IgG depleted sampled. This will typically be fractions 2 - 4.

Protocols (cont.)

IgG Purification

1. Dilute sample (serum, plasma, ascites fluid, or tissue culture supernatant) 1:1 with Protein G Binding Buffer before applying to the Protein G affinity column.

   Note: Plasma samples will become cloudy/opaque after dilution with the Protein G Binding Buffer. This is a result of lipoprotein precipitation. If this occurs, centrifuge the sample at 10,000 x g for 15 minutes (or until clear).
2. Transfer the Protein G affinity column to a second test tube and add the diluted sample to the Protein G affinity column.
3. Allow sample to flow through the Protein G affinity column.
4. Collect the filtrate solution in a test tube and set aside for analysis of binding efficiency.
5. Pipette 10 mL of Protein G Binding Buffer to wash the Protein G affinity column and allow it to pass through the affinity column.
6. To verify that all unbound proteins are washed away, collect filtrate in 2 mL fractions. Measure their absorbance readings at 280 nm. The last fractions should have absorbance readings that are the same as the Protein G Binding Buffer.

IgG Elution

1. Pipette 6 mL of Elution Buffer to the Protein G affinity column to recover the bound IgG.
2. Collect the filtrate in numbered collection tubes. For collection purposes, it is important to know where in the column your sample is. The gel bed holds approximately 2 mL of solution. If you have a 2 mL of sample, then the entire sample will fit into the gel bed. As the sample passes through the column, the first 2 mL that passes through the column will contain some of the Elution Buffer and some of the purified IgG. The next fractions will contain the majority of the purified IgG.

   Note: We recommend that you collect the sample in 0.5 mL to 1.0 mL fractions.

1053_88391B 12/9/05 2:36 PM Page 16

Protocols (cont.)

Sample Neutralization

1. Prepare a high ionic strength alkaline neutralization buffer. 1M Phosphate, pH 7.5 - 9.0 or 1M Tris, pH 7.5 - 9.0 are sufficient.
2. If using a neutralization buffer with a pH of 7.5, add 100 µL to each 1 mL fraction. If using a neutralization buffer with a pH of 9.0, add 50 µL to each 1 mL fraction.

Purified IgG Identification

1. Blank a spectrophotometer set at 280 nm. You can use deionized water or 1 mL of Protein G Elution Buffer with 50 - 100 µL of neutralization buffer to blank the spectrophotometer.
2. Measure the absorbance of each fraction at the 280 nm reading.
3. The fractions with the highest absorbance readings will contain your purified IgG.
4. Pool only the purified fractions with the highest absorbance readings.

Protein G Affinity Column Regeneration

1. To regenerate the column, wash the Protein G affinity column with 5 mL of Protein G Elution Buffer. To store the Protein G affinity column, equilibrate it with 5 mL of Binding Buffer with 0.02% sodium azide. Recap the column when 1 mL of buffer remains above the disc at the top of the column. Recap column by replacing bottom cap first. Store upright at 4 °C.

Protocols (cont.)

Sample Desalting

We recommend that a separate desalting column be used for every 1 mL sample.

1. Bring desalting columns to room temperature. It is important that the column, sample and buffer are all at the same temperature.
2. Prepare 100 mL of desalting buffer.
3. Remove the top cap from the desalting column and pour off storage solution.
4. Remove the bottom cap of the desalting column and add 10 mL of desired desalting buffer and allow it to pass completely through the desalting column.
5. Prepare a series of collection tubes (test tubes) numbered 1 - 10.
6. Pipette 1 mL of sample to the desalting column and collect the first 1 mL fraction into the first numbered collection tube.
7. When the sample drains down to the disc at the top of the gel bed, pipette desalting buffer to the column and continue collecting the sample in 0.5 mL fractions. Collect each 0.5 mL fraction in separate collection tubes.
8. To identify which fractions contain the desalted, purified IgG, measure the absorbance reading at 280 nm of each fraction, comparing it to the desalting buffer.
9. The fractions with the high absorbance values can then be pooled.
10. After collection of the antibody, wash the desalting column with an additional 15 mL of desalting buffer with 0.02% sodium azide. Cap the desalting column when approximately 2 mL of buffer remains above the disc at the top of the column. Store the column upright at 4 °C.

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Protocols (cont.)

Protocol for IgG and Albumin Removal from plasma/serum samples
The IgG Purification Kit should be used first since it can process a larger volume sample than the Albumin Depletion Kit. The binding capacity of the affinity columns in the Enchant IgG Purification Kits allows for removal of IgG from 1 to 2 mL of plasma/serum. The total IgG content of serum is typically 10 - 15 mg/mL, while the specific IgG of interest only accounts for 2 - 5% of this total. Albumin is the most abundant protein in serum at 30 - 40 mg/mL.

IgG Depletion
Protein A or Protein G IgG Purification Kits can be used depending on the species the sample was isolated from and the antibody isotype that needs to be depleted.

Note: All components should be at same temperature otherwise bubbles could form in the column and prevent flow.

1. Dilute 1 mL of sample 1:1 with Binding Buffer before applying to the column.

   Note: Plasma samples will become cloudy/opaque after dilution with the Binding Buffer. This is a result of lipoprotein precipitation. If this occurs, centrifuge the sample at 10,000 x g for 15 minutes (or until clear). Apply cleared supernatant to the Protein A or G affinity column.
2. Remove storage buffer from column and wash column with 5 mL Binding Buffer. Allow buffer to pass through column by gravity flow.
3. Add diluted sample to column and allow it to flow through column.
4. Collect 1 mL fractions and set aside for analysis. Wash column by passing 10 - 15 mL of Binding Buffer through column by gravity flow.
5. Collect 1 mL fractions and set aside for analysis

   Note: Typically the IgG depleted sample is distributed between 4 flow-through fractions. Fractions with highest protein content can be pooled together and saved for albumin depletion. The concentration of albumin in these pooled fractions is typically around 10 mg/mL.
6. The bound IgG can be eluted from column using Elution Buffer supplied with kit. Eluted fraction can consequently be neutralized with alkaline buffers and desalted using the desalting columns provided with the kit. By measuring absorbance at 280 nm of the eluted IgG fractions we can quantify the amount of IgG that was removed by the column.
7. Columns can be regenerated and reused.

Protocols (cont.)

Albumin Depletion

1. Place one albumin depleting disc into a Nanosep® centrifugal device.
2. Add 380 mL of sterile water to the Nanosep centrifugal device containing the albumin depleting disc. Vortex for 5 seconds.
3. Centrifuge Nanosep albumin column at 12,000 x g for 1 minute. Discard the filtrate.
4. Apply 100 µL (containing approximately 1 mg of albumin) of the IgG depleted sample (saved from step 5) to the albumin depletion column.

   Note: Flow-through fractions can be desalted through buffer exchange using Nanosep spin devices prior to albumin depletion but this step is not necessary.
5. Incubate sample in the column for 2 minutes.
6. Centrifuge the column at 12,000 x g for 1 minute. Retain filtrate. This will contain IgG and albumin depleted sample.
7. Retained filtrate fractions can now be analyzed by SDS-PAGE and 2D analysis.
8. The bound albumin can be eluted from the column using high-salt solutions or gel loading buffers.

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Troubleshooting

Problem	Cause	Solution
Slow flow or no flow of Protein A or Protein G affinity columns (< 0.5 mL/min)	• Antibody sample was not clarified prior to applying to affinity column (e.g., lipoprotein precipitation) • Buffers or antibody sample outgassed in the affinity column. This results in air bubbles within column, preventing flow	• Centrifuge diluted sample to sediment particulate matter • De-gas buffers and remove air bubbles from column
Antibody is purified, but does not have function	• Antibody is sensitive to low-pH Elution Buffer	• Use a neutral Elution Buffer, pH 6.6
Antibody is not detected in eluted fractions when read at 280 nm absorbance readings	• Antibody sample did not elute • Used desalting column instead of Protein A or Protein G affinity column • Antibody sample does not contain antibody species or isotype that binds either to Protein A or Protein G	• Use a neutral Elution Buffer, pH 6.6 • Re-apply sample to affinity column • Ensure sample contains IgG type antibody (e.g., ELISA). • Use charts on pg 5 - 7 to ensure proper affinity column is selected
Sample is not depleted of IgG	• Amount of IgG in sample exceeded binding capacity of affinity columns	• Reduce starting sample volume to 1 - 2 mL. Dilute sample volume 1:1 in Binding Buffer

Complementary Products

- **Enchant™ Albumin Depletion Kits** offer high capacity albumin depletion in just 10 minutes.
- **Pall Life Sciences offers Centrifugal Devices** for processing the following sample volumes:

Device	Sample Volume
Nanosep®	50 µL to 500 µL
Microsep™	0.5 mL to 3.5 mL
Macrosep®	3.5 mL to 15 mL
Jumbosep™	15 mL to 60 mL

- **Nanosep MF (microfiltration) Centrifugal Devices** are available with Bio-Inert® or GHP membranes for low protein-binding and high recoveries in applications such as particulate removal prior to sample analysis (GHP product is HPLC grade) and removal of precipitates.
- **BioTrace™, Biodyne® and FluoroTrans® Transfer Membranes** offer precise performance and compatibility with nearly every detection system available.
- **AcroPrep™ and AcroWell™ Filter Plates** offer superior performance for high throughput sample preparation and detection procedures.
- **BTS-SP Media** is a highly asymmetric membrane engineered for serum separation from whole blood.
- **Acrodisc® Sterile Syringe Filters** are available in a variety of diameters, membranes and pore sizes to meet virtually all sample preparation needs.
- **BioSepra® Chromatography Resin** for the purification of biomolecules and compounds. Available chemistries include Affinity, Ion Exchange, Size Exclusion, Hydrophobic Interaction and Hydroxyapatite.

WARNING

Employment of the products in applications not specified, or failure to follow all instructions contained in this product information insert, may result in improper functioning of the product, personal injury, or damage to property or the product. See Statement of Warranty in our most recent catalog.

1053_88391B 12/9/05 2:36 PM Page 22

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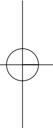
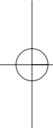
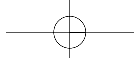
1053_88391B 12/9/05 2:36 PM Page 23







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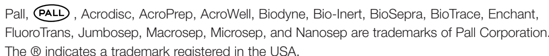

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