Life Sciences

Enchant™ Albumin Depletion Kit

• High Capacity Albumin Depletion In Just 10 Minutes

Ordering Information

Prod. No.	Description	Pkg
5300-ALBDEP	Enchant Albumin Depletion Kit	25 purifications

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PN 88390B

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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.*

Introduction

The Enchant™ Albumin Depletion Kit contains all of the necessary components to process 10 - 100 µL of serum or plasma per sample. The albumin depleting discs utilize a Cibacron* Blue based support that is re-hydrated to form a gel based slurry. The gel based slurry is equivalent to 200 µL of resin. The easy five step protocol allows you to remove 2 mg of albumin from each sample processed.

The Enchant Albumin Depletion Kit was optimized for use with human serum and plasma samples. In addition, the kit can be optimized for use with other species including bovine, calf, goat and rat. The kit is not recommended for use with mouse.

Specifications

Binding Capacity
Each albumin depleting disc can remove > 2 mg of albumin from diluted serum or plasma samples.

Storage Conditions
Store at room temperature.

Shelf Life
One year from date of purchase, if stored properly.

Enchant™ Albumin Depletion Kit Components
Nanosep® Centrifugal Device
Quantity: 25 complete columns, 25 additional filtrate tubes
Filter Media: 0.45 µm GHP Membrane (hydrophilic polypropylene)
Sample Reservoir: Polypropylene
Membrane Support Base: Polypropylene
Filtrate Receiver: Polypropylene

Centrifugal Device - Dimensions
Outside Diameter (maximum): fits 1.5 mL microcentrifuge tube rotors
Overall Length (fully assembled): 4.5 cm (1.77 in.)

Maximum Centrifugal Force
14,000 x g

Albumin Depleting Discs
Quantity: 25 discs
Cibacron* Blue based dehydrated support.

Binding/Wash Buffer
Quantity: 6.25 mL

Frequently Asked Questions

Q: Do I need to store the Enchant™ Albumin Depletion Kit in a cold room?

A: No. The Enchant Albumin Depletion Kit can be stored at room temperature. The kit should not be frozen.

Q: Does pH effect the binding of the Enchant albumin depleting spin column?

A: Yes. The optimal pH range for albumin binding is between 6.0 - 9.0.

Q: Do I need to desalt my sample prior to using the Enchant Albumin Depletion Kit?

A: Yes. Binding of albumin to the resin is salt dependent. The samples have to be desalted prior to applying to the albumin depleting spin column. The Nanosep® ultrafiltration centrifugal devices offer a superior platform for sample desalting (see Complementary Products section). If dialysis is preferred, we recommend the following buffers; human and swine samples (25 mM Tris, 75 mM NaCl, pH 7.5), for bovine, goat, calf and rat samples (25 mM Tris, 25 mM NaCl, pH 7.5). This kit is not recommended for mouse species.

Q: Can the same wash/binding buffer be used for all species?

A: No. The Enchant Albumin Depletion Kit was optimized for the removal of albumin from human serum and plasma samples. Depending on the species, a different wash/binding buffer may be needed. For bovine, calf and goat species, we recommend a wash/binding buffer of 25 mM Tris pH 7.2.

Q: Does my sample need to be free of particulate matter prior to adding to Nanosep albumin depleting spin column?

A: Yes. For optimal performance of the Enchant Albumin Depletion Kit, the serum or plasma sample should be clarified by microfiltration. The Nanosep microfiltration centrifugal devices provide a convenient platform for removing particulates prior to processing (see Complementary Products section).

Protocols

These protocols are intended to be used as guidelines. Depending on your application, further optimization may be necessary.

Serum Sample Preparation

It is important not to exceed the binding capacity of each albumin depleting disc. Each disc will bind > 2 mg of human serum albumin and requires a load volume of at least 50 µL. For highly concentrated serum or plasma samples, dilute sample 10 - 100 µL with the binding/wash buffer. For dilute samples, > 50 µL can be applied directly to albumin depleting spin column without dilution. If using species other than human, refer to FAQ section for buffer guidelines.

1. Place one albumin depleting disc into a Nanosep® centrifugal device.
2. Add 380 µL of sterile water to each Nanosep centrifugal device containing the albumin depleting disc. Vortex for five seconds. The disc should be fully hydrated in less than 30 seconds.
3. Place the Nanosep albumin depleting spin column in a centrifuge and centrifuge at 12,000 x g for one minute.
   Discard the filtrate.
   Note: Make sure that you counterbalance the centrifuge.
4. Apply between 50 and 100 µL of diluted albumin containing sample. Typical sample volumes have a total volume of 100 µL but only contain ≤ 30 µL of serum.
   Note: When sample is added to the Enchant™ albumin depleting spin column, a slurry does not form, it just becomes wet.
5. Incubate sample in the albumin depleting spin column for 2 minutes.
6. Centrifuge the albumin depleting spin column at 12,000 x g for one minute. Recover filtrate and add back to the albumin depleting spin column.
   Note: Make sure that you counterbalance the centrifuge.
7. Incubate sample in the albumin depleting spin column for another 2 minutes.

Protocols, Serum Sample Preparation (cont.)

8. Centrifuge the albumin depleting spin column at 12,000 x g for one minute. Retain the filtrate for downstream analysis. Replace used Nanosep® filtrate tube with a new filtrate tube.
   Note: Make sure that you counterbalance the centrifuge.
9. Add 50 µL of the binding/wash buffer to the Enchant™ albumin depleting spin column. This will wash through any unbound proteins.
10. Centrifuge the albumin depleting spin column at 12,000 x g for one minute. Retain filtrate.
    Note: Make sure that you counterbalance the centrifuge.
11. Analyze retained filtrate fractions by SDS-PAGE.
12. Dispose of spin column, do not re-use for another sample.

Note: A single wash of the Enchant albumin depleting spin column is typically sufficient for obtaining adequate amounts of the desired protein sample. If further washes are needed, repeat steps 9 and 10 as needed and combine desired fractions. If additional washes are needed, you can order Nanosep filtrate tubes PN FD001X34.

Recovering Bound Albumin

You can elute the bound albumin from the resin with the following protocol.

1. Add 200 µL of any gel loading buffers diluted to 1X (Example: 0.02 M sodium phosphate, 0.5 M sodium thiocyanate, pH 7.2). to the albumin depleting spin column. High salt concentrations will also remove the bound albumin (Example: 500 mM salt solution).
2. Centrifuge at 12,000 x g for one minute.
   Retain the filtrate.
   Note: Make sure that you counterbalance the centrifuge.
3. Repeat steps 1 and 2 if desired. Replace filtrate tube each time.
4. Analyze filtrates by SDS-PAGE.
5. Dispose of column, do not re-use for another sample.

Protocols (cont.)

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 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 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.

Protocols, IgG Depletion (cont.)

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.

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.

Troubleshooting

If high levels of albumin were visualized on the SDS-PAGE gel, a variety of factors could have caused inefficient removal of the albumin.

Factors effecting albumin depletion:

Possible Cause	Possible Solution
Salt concentration of sample is high	Desalt sample with Nanosep® ultrafiltration centrifugal devices
Concentration of albumin in sample exceeds the binding capacity of the albumin depleting disc	Dilute sample prior to applying to Enchant™ albumin depleting spin column
Sample type is incompatible with Enchant Albumin Depletion Kit	The Enchant Albumin Depletion Kit was optimized for human serum and plasma sample. Although other species of serum/plasma can be used, not all species are compatible.
Serum or plasma sample prep method is incompatible	• Prepare serum or plasma by alternative method • Dialyze sample with low salt buffer

Complementary Products

- Pall Life Sciences offers ultrafiltration centrifugal devices for processing the following sample volumes:

Device	Sample Volume
Nanosep® Device	up to 0.5 mL
Microsep™ Device	0.5 mL to 3.5 mL
Macrosep® Device	3 mL to 15 mL
Jumbosep™ Device	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.
- **Enchant™ IgG Purification and Depletion Kits** offer simple, easy to use Protein A or Protein G affinity columns for IgG purification or depletion.
- **Acrodisc® Sterile Syringe Filters** are available in a variety of diameters, membranes and pore sizes for meeting 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.

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