Life Sciences

Enchant™ Multi-Protein Affinity Separation Kit

Description

The Enchant Multi-Protein Affinity Separation Kit includes all of the components necessary to fractionate albumin and IgG from 50 µL of normal human serum or plasma under native or denatured conditions. The Anti-HSA and IgG Fractionation Resins utilize unique ligands coupled to a solid support resulting in highly specific removal of the target protein(s). The ability to process samples under denatured conditions further increases the specificity of the kit because the presence of a strong denaturant breaks any protein:protein interactions and provides greater confidence that only the target proteins are removed.

This kit is designed for single use which eliminates the possibility of sample cross contamination due to reuse of the device/column. The centrifugal devices included in the kit provide for a fast and efficient removal of ~80% of the total protein content found in serum or plasma, allowing for easier identification of moderate to low abundant proteins and biomarkers. Additionally, if the bound proteins need to be analyzed downstream, they can be eluted from the resin for analysis.

Ordering Information

Prod. No.	Description/Connections	Packaging
5300-AFFMPS	Enchant Multi-Protein Affinity Separation Kit for Human Albumin and IgG	24 samples

Specifications

Binding Capacity
> 97% removal of albumin and IgG from 50 µL of serum/plasma diluted 1:5

Maximum Sample Volume
50 µL

Sample Type and Species
Human serum or plasma

Specificity
> 90% of proteins bound during fractionation will be target protein when the eluate is measured by SDS-PAGE

Storage Conditions
Store at 4 °C

Shelf Life
One year from date of purchase if stored as directed.

Enchant Multi-Protein Affinity Separation Kit Components
Nanosep® Centrifugal Device
Quantity: 24 complete columns
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 rotors
Overall Length (fully assembled): 4.5 cm (1.8 in.)

Recommended Centrifugal Force
800-900 x g (3,000 rpm)

Anti-HSA Fractionation Resin
Volume: 10 mL
Resin is supplied in an aqueous slurry containing 0.02% sodium azide.

Anti-IgG Fractionation Resin
Volume: 10 mL
Resin is supplied in an aqueous slurry containing 0.02% sodium azide.

Binding/Wash Buffer
Volume: 20 mL per bottle
pH: 7.4

Instructions for Use

Sample Preparation

1. To process samples under denatured conditions, use the following sample preparation steps:
   a. Make a fresh batch of 9M urea + 2% CHAPS in Tris, pH 9.0, using high quality water. Alternatively, aliquots can be stored at –20 °C. Older preparations of urea stored at 4 °C or room temperature can result in carbamylation of proteins during sample handling which makes MS analysis much more difficult.
   b. Each Nanosep spin column included in the kit is designed for the depletion of albumin and IgG from 50 µL of serum or plasma. To this amount of sample add 63 µL 9M urea + 2% CHAPS in Tris ([urea] = 5M). Incubate at room temperature for 30 minutes.
   c. Add 136 µL of Binding/Wash Buffer for a final volume of 250 µL (final [urea] = 2.25 M).
   d. Once denatured, keep samples at room temperature until they are loaded into the Nanosep centrifugal device packed with the Fractionation Resin(s).

Instructions for Use (cont.)

2. To process samples under native conditions, use the following sample preparation steps:
   a. Dilute 50 µL of serum or plasma 5-fold in Binding/Wash Buffer. To dilute 5-fold, add 200 µL of Binding/Wash Buffer to 50 µL of serum or plasma.
   b. Following dilution, keep the samples on ice until they are loaded into the Nanosep® centrifugal device packed with the Fractionation Resin(s).

Note: Visually inspect samples prior to dilution. If there is significant precipitate in the sample a filtration or centrifugation step is recommended prior to fractionation (see chart below for product recommendation). This can be done before or after sample dilution. The overall performance and reproducibility of the fractionation and other downstream methods will be improved if particulates are removed.

Sample Volume	Device	Part Number
1 – 150 mL	Serum Acrodisc®	4525
50 – 500 µL	Nanosep Centrifugal Device	ODGHPC34
Up to 300 µL	AcroPrep™ 96 Filter Plate	5030

Serum and plasma samples do not go through freeze-thaw cycles well. There are changes that occur in the proteins as well as an increased incidence of cryoprecipitates. It is recommended that samples be aliquoted to minimize freeze-thaw cycles if they will be used more than once.

Single Protein Fractionation (IgG or HSA)

1. Ensure that the Anti-IgG or HSA Fractionation Resin is in a homogenous suspension prior to pipetting into the Nanosep centrifugal device. To minimize the formation of bubbles in the bottle, we recommend gently rolling the bottle between the palms of your hands.
2. Pipette 400 µL of the appropriate Fractionation Resin into a Nanosep centrifugal device. Centrifuge at 800 - 900 x g (3,000 rpm) for 2 minutes and discard flow through storage buffer.
   Note: We recommend that you draw 400 µL of resin up into the pipet tip and dispense back into the bottle. Then, draw another 400 µL up into the pipet tip and dispense into the Nanosep Centrifugal Device.
3. Wash 1 time with Binding/Wash Buffer. Add 400 µL of Binding/Wash Buffer and vortex. Centrifuge at 800 - 900 x g (3,000 rpm) for 2 minutes and discard flow through wash solution
4. Add 250 µL of denatured or native sample to the resin-packed Nanosep centrifugal device.
5. Mix well by vortexing and then tumble end over end for 15 minutes at room temperature.
6. Centrifuge at 800 - 900 x g (3,000 rpm) for 1.5 minutes and collect flow through. The flow through is your albumin or IgG depleted sample.
7. If you are interested in collecting loosely retained proteins, collect several wash fractions for analysis. Washing is also recommended if eluates will be analyzed. Wash the resin 3 - 4 times with 400 µL of Binding/Wash Buffer. Mix/vortex after each buffer addition. You can use an A280 measurement after last wash to ensure that no more protein is being washed off.
8. To elute the bound protein, add 400 µL of 0.1 M Glycine pH 2.3 (not included in the kit).
9. Mix well by vortexing and tumble end over end for 15 minutes at room temperature.
10. Centrifuge at 800 - 900 x g (3,000 rpm) for 1.5 minutes and collect the eluate. The bound protein will be present in the eluate fraction.
11. To neutralize eluate add 40 µL (1/10 volume) of 1M Tris-HCl pH 8 to bring pH back to neutral (~pH 7).
12. For improved recovery, repeat the elution steps 7 - 10 one more time.

Multi-Protein Fractionation (IgG and HSA)

1. Ensure that the Anti-IgG and HSA Fractionation Resins are in a homogenous suspension prior to pipetting into the Nanosep centrifugal device. To minimize the formation of bubbles in the bottle, we recommend gently rolling the bottles between the palms of your hands.
2. Pipette 400 µL of the IgG Fractionation Resin into a Nanosep centrifugal device. Centrifuge at 800 - 900 x g (3,000 rpm) for 2 minutes and discard storage buffer.
   Note: We recommend that you draw 400 µL of resin up into the pipet tip and dispense back into the bottle. Then, draw another 400 µL up into the pipet tip and dispense into the Nanosep Centrifugal Device.
3. Pipette 400 µL of the HSA Fractionation Resin into the same Nanosep centrifugal device. Centrifuge at 800 - 900 x g (3,000 rpm) for 2 minutes and discard storage buffer.
   Note: We recommend that you draw 400 µL of resin up into the pipet tip and dispense back into the bottle. Then, draw another 400 µL up into the pipet tip and dispense into the Nanosep Centrifugal Device.
4. Wash 1 time with Binding/Wash Buffer. Add 400 µL of Binding/Wash Buffer and vortex. Centrifuge at 800 - 900 x g (3,000 rpm) for 2 minutes and discard flow through (wash solution).
5. Add 250 µL of denatured or native sample to the resin-packed Nanosep centrifugal device.
6. Mix well by vortexing and then tumble end over end for 15 minutes at room temperature.

Instructions for Use (cont.)

7. Centrifuge at 800 - 900 x g (3,000 rpm) for 1.5 minutes and collect the flow through. The flow through is the albumin and IgG depleted sample.
8. If you are interested in collecting loosely retained proteins, collect several wash fractions for analysis. Washing is also recommended if eluates will be analyzed. Wash the resin 3 - 4 times with 400 µL of Binding/Wash Buffer. Mix/vortex after each buffer addition. You can use an A280 measurement after last wash to ensure that no more protein is being washed off.
9. To elute the bound proteins, add 400 µL of 0.1 M Glycine pH 2.3 (not included in kit).
10. Mix well by vortexing and tumble end over end for 15 minutes at room temperature.
11. Centrifuge at 800 - 900 x g (3,000 rpm) for 1.5 minutes and collect the eluate. The bound proteins will be present in the eluate fraction.
12. To neutralize the eluate, add 1/10 volume of 1M Tris-HCl pH 8 to bring pH back to neutral (~pH 7).
13. For improved recovery, repeat the elution steps 7 - 10 one more time.

Complementary Products

- **AcroPrep™ Filter Plates** offer superior performance for high throughput sample preparation and detection procedures.
- **Pall Life Sciences offers centrifugal spin filters** for sample concentration and desalting in 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	3.5 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), removal of precipitates or as a housing to easily manipulate the processing of bead bound targets.
- **BioTrace™, Biodyne® and FluoroTrans® Transfer Membranes** offer precise performance and compatibility with nearly every detection system available.
- **Enchant™ IgG Purification and Depletion Kits** offer simple, easy to use Protein A or Protein G Affinity columns for IgG pruification or depletion.
- **Enchant Albumin Depletion Kits** offer high capacity albumin depletion in just 10 minutes.
- **BioSepra® Chromatography Resin** for the purification of biomolecules and compounds. Available chemistries include Affinity, Ion Exchange, Size Exclusion, Hydrophobic Interaction, and Hydroxyapatite.
- **BTS - SP Media** is a highly asymmetric membrane engineered for serum separation from whole blood.

WARNING

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