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Product Information

HT Seppro® IgY14
96 Well Plate

Catalog Number **S2453**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The HT Seppro® IgY14 Plate is specifically designed to remove fourteen highly abundant proteins from human serum or plasma. The product is based on avian antibodies (IgY) immobilized on a resin and incorporated into a 96 well plate for high throughput depletion. The following proteins are depleted in a single step:

Albumin	IgG
α_1-Antitrypsin	IgA
IgM	Transferrin
Haptoglobin	α_2-Macroglobulin
Fibrinogen	Complement C3

α_1-Acid Glycoprotein (Orosomucoid)
HDL (Apolipoproteins A-I and A-II)
LDL (mainly Apolipoprotein B)

Selective immunodepletion provides an enriched pool of low abundance proteins for downstream proteomic analyses. Specific removal of these fourteen highly abundant proteins depletes ~95% of the total protein mass from human serum or plasma, thereby allowing the low abundance proteins in the flow-through fractions to be studied via liquid chromatography/mass spectrometry (LC/MS).

Characteristics of HT Seppro® IgY14 Plates
The resin can be used 10 times with no loss of efficiency in performance. Before using the plate for samples, it is suggested to run one or two blank samples (buffer only) to remove any residual non-covalently bound IgY from the beads. **It is recommended to perform only one depletion cycle per day.**

The resin has been shown to have a capacity of 85 µg total protein per well. Based on pooled normal human plasma or serum at a concentration of ~50 mg/mL, 1.5–2.0 µL of sample can be loaded per well (diluted in a total volume of 50 µL). To ensure optimal depletion it may be necessary to load less protein mass for samples, which contain higher levels of certain abundant proteins. There is a maximum volume load to ensure the entire sample is loaded into the resin bed. (See Preparation Instructions)

Total protein mass removal: ~95%

Targeted depletion efficiency: >99% for albumin, 95% average for remaining 13 targets

Operating temperature: 20–25 °C

Shipping Buffer: 1× Dilution Buffer with 0.02% sodium azide

Components

HT Seppro® IgY14 Plate (Catalog Number S2453)	1 each

Reagents and Equipment Required but Not Provided.

10× Ammonium Bicarbonate Buffer
2 M Ammonium Bicarbonate, pH 8.2
(Catalog Number S2454)

10× Dilution Buffer
Tris-Buffered Saline (TBS) - 100 mM
Tris-HCl with 1.5 M NaCl, pH 7.4
(Catalog Number S4199)

10× Stripping Buffer
1 M Glycine-HCl, pH 2.35
(Catalog Number S4324)

10× Neutralization Buffer
1 M Tris-HCl, pH 8.0
(Catalog Number S4449)

96 well Sample Collection Plate, V-bottom
Corning® 96 well storage system
(Catalog Number CLS3363)

96 well deep well Wash Plate
Nunc® 96 DeepWell™ plate or equivalent
(Catalog Number P8241)

Multiwell Plate Vacuum Manifold
Pall® AcroPrep™ 96 well multiwell plate manifold
(Catalog Number Z722316)
350 µL receiver plate spacer block (collection)
1 mL receiver plate spacer block (wash)
(Pall Catalog Number 5014)

Aluminum Adhesive Seal
AlumaSeal® II film
(Catalog Number A2350)

Corning® Costar® Spin-X® Centrifuge Tube Filters
0.45 µm, pack of 100
(Catalog Number CLS8163)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Preparation of 1× concentration buffers
Note: Use ultrapure water for preparation of 1× buffers.

Separately dilute the four 10× buffers (Ammonium Bicarbonate, Dilution, Stripping, and Neutralization Buffers) 10-fold with ultrapure water. If precipitation is observed in any of the 10× buffers, allow the bottle to warm to room temperature and mix until completely dissolved prior to use.
Note: Do not dilute the entire bottle of 10× Neutralization Buffer. If analysis of bound (depleted) proteins is desired, it is necessary to save a volume of the10× Neutralization Buffer for neutralization of this fraction following elution.

Sample Preparation
It is not recommended to load unfiltered plasma or serum directly onto the plate. Dilute a serum/plasma sample in 1× Ammonium Bicarbonate Buffer to a final concentration of <85 µg/50 µL.
Note: It is suggested to **avoid using reducing reagents, such as DTT or BME, or denaturing reagents, such as urea or guanidine-HCl in the sample extracts**. Remove particulates with a 0.45 µm Costar Spin-X centrifuge tube filter by centrifuging for 1 minute at 10,000 × *g*.

Plate Preparation
It is recommended to centrifuge the plate for 2 minutes at 1,000 × *g* before removing the seals to recover any shifting of resin during shipment.

Storage/Stability
Store the HT Seppro® IgY14 Plate at 2–8 °C. **Do Not Freeze** the plate.

After use, seal the bottom of the plate with AlumaSeal II film, add 150 µL of 1× Dilution Buffer containing 0.02% sodium azide, seal the top of the plate with AlumaSeal II film, and store the plate at 2–8 °C.

Procedure
Notes: Before using the plate for samples, it is suggested to run one or two blank samples (buffer only) to remove any residual non-covalently bound IgY from the beads.

It is recommended to use a vacuum pump with vacuum adjusted to 10–12 in. Hg (4.9–5.9 PSI).

Use the thin 1 mL receiver plate spacer block with the deep well wash plate and the thick 350 µL receiver plate spacer with sample collection plates to ensure proper collection of flow-through.

Immunocapture of Serum/Plasma Protein(s)
1. Apply vacuum to the plate for 20 seconds to remove excess buffer.
2. Equilibrate resin 5 times with 100 µL of 1× (200 mM) Ammonium Bicarbonate buffer. Apply vacuum to the plate for 20 seconds between each buffer load to remove excess buffer.
3. Check bottom of plate for hanging droplets and tap off. It is recommended to quickly blot the bottom of plate before loading sample.

4. Immediately add 50 µL of sample per well (containing up to 85 µg total protein, diluted in 1x Ammonium Bicarbonate buffer). Sample should be loaded slowly on top of the resin bed in each well. Avoid applying samples down the side of well, or with enough force to cause bubbles or channel formation within the resin.
5. Cover the plate with AlumaSeal II film to prevent evaporation. Allow the sample to incubate with the resin for 1 hour at room temperature on the benchtop or on vacuum manifold with a collection plate beneath the filter plate.
   Note: Do not shake.
6. Collect flow-through sample in the collection plate by applying vacuum to the plate for 20 seconds.
7. Collect a flow-through wash directly into well containing flow-through sample by applying 100 µL of 1× Ammonium Bicarbonate buffer to the resin bed. Apply vacuum to the plate for 20 seconds. Total sample volume collected should be equal to 150 µL. The sample is now ready for downstream analysis.

Elution of Bound Protein(s)

1. To remove proteins non-specifically bound to beads, wash beads 3 times with 100 µL of 1× Dilution Buffer. Apply vacuum to the plate for 20 seconds between each wash to remove excess buffer. Equilibration in 1× Dilution Buffer will minimize precipitation and gas production upon pH change with glycine buffer.
2. Strip bound proteins from resin bed with 5 washes with 100 µL of 1× Glycine Stripping Buffer. Allow stripping buffer to incubate with the beads for 2 minutes for each wash.
3. Apply vacuum to the plate for 20 seconds between each wash to remove excess buffer.
   Note: Do not allow this step to take more than 15 minutes total time. It is crucial for resin stability to immediately neutralize the beads. (see Regeneration of Plate Resin). These elution fractions may be collected and analyzed as desired.

Regeneration of Plate Resin

1. To regenerate the plate after stripping bound proteins, immediately neutralize the beads by adding 100 µL of 1× Neutralization Buffer.
2. Apply vacuum to the plate for 20 seconds to remove excess buffer.
3. Add an additional 100 µL of 1× Neutralization buffer and incubate at room temperature for 5 minutes.
4. Apply vacuum to the plate for 20 seconds to remove excess buffer.
5. Wash the beads 3 times with 100 µL of 1× Dilution Buffer.
6. Apply vacuum to the plate for 20 seconds between each wash to remove excess buffer.
7. Blot dry and seal the bottom of the plate with AlumaSeal II film.
8. Add 150 µL of 1× Dilution Buffer containing 0.02% sodium azide.
   Note: Do not apply vacuum. Seal the top of the plate with AlumaSeal II film and store at 2–8 °C until next depletion.

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