

# High-Select™ HSA/Immunoglobulin Depletion Resin

Catalog Numbers A36365, A36366, A36367, and A36368

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## Contents

Cat. No.	Product	Contents	Storage
A36365	High-Select™ HSA/Immunoglobulin Depletion Mini Spin Columns	6 columns Each column contains 200 µL of a 50% slurry in 10mM PBS, 0.02% sodium azide, pH 7.4	Store at 4°C. Do not freeze.
A36366	High-Select™ HSA/Immunoglobulin Depletion Mini Spin Columns	24 columns Each column contains 200 µL of a 50% slurry in 10mM PBS, 0.02% sodium azide, pH 7.4	
A36367	High-Select™ HSA/Immunoglobulin Depletion Midi Columns	10 columns Each column contains 400 µL of a 50% slurry in 10mM PBS, 0.02% sodium azide, pH 7.4	
A36368	High-Select™ HSA/Immunoglobulin Depletion Resin	50 mL 50% slurry in 10mM PBS, 0.02% sodium azide, pH 7.4	

## Product description

The Thermo Scientific™ High-Select™ HSA/Immunoglobulin Depletion Resin and columns are used for the removal of human serum albumin (HSA) and immunoglobulin from human serum and plasma. This depletion resin uses highly specific immobilized anti-HSA and anti-immunoglobulin (IgG, IgA, IgD, IgE, IgM) antibodies for protein removal, providing minimal nonspecific interactions with other proteins.

Analysis of human fluids is often complicated by the presence of high concentrations of albumin and IgG that represent more than 70% of the total serum protein. Removal of these proteins is often essential for the study of low-abundant proteins. The High-Select™ HSA/Immunoglobulin Depletion Resin can deplete >95% of HSA and >95% of IgG from 10 µL of plasma or serum with the mini columns or 100 µL of plasma or serum with the midi columns within ~10 minutes. Alternatively, researchers may customize with the bulk resin to suit experimental needs. The depletion of high-abundant proteins enables the identification and quantitation of low-abundant proteins in samples by mass spectrometry (MS).

## Additional information

- The depletion spin columns contain a storage solution used as a dilution and binding buffer for direct processing of serum or plasma.  
**Note:** No other additions or solvent exchanges are required before protein depletion.
- The mini depletion spin columns can process a maximum of 10 µL of human serum or plasma. The midi depletion spin columns can process a maximum of 100 µL of human serum or plasma. For serum samples containing abnormally high amounts of albumin or IgG, the sample load may need to be reduced.
- The depletion spin columns are designed for single use. **Do not reuse the resin.**
- The depletion resin is designed for use with human serum or plasma samples and has not been tested on any other species. Alternative human biological fluids (e.g., cerebrospinal fluid or amniotic fluid) may be used with this product but may require optimization.
- The High-Select™ HSA/Immunoglobulin Depletion Resin allows the user to customize the fill of resin depending on experimental needs. This resin is designed to only be used once per sample. **Do not reuse the resin.**

## Additional materials required

- Microcentrifuge and centrifuge capable of operating at 1000 × g
- 2 mL collection tubes
- 15 mL collection tubes
- End-over-end mixer

## Remove HSA/IgG from samples

Because of the high concentration albumin present in serum, each mini column (100 µL of antibody resin bed) binds sufficient albumin and IgG to process up to 10 µL (600 µg) of serum or plasma and each midi column (200 µL of antibody resin bed) binds up to 100 µL (6,000 µg) of serum or plasma. However, the amount of HSA and IgG in serum or other fluid samples will vary considerably. For best results, optimize the ratio of sample to slurry volume for each specific application.

## Remove HSA/IgG in mini format

1. Equilibrate the depletion spin column to room temperature.
2. Remove the column screw cap and add up to 10  $\mu$ L of sample directly to the resin slurry in the column.
3. Cap the column and invert the column several times until the resin is completely homogenous in solution.
4. Incubate the mixture in the column with gentle end-over-end mixing for 10 minutes at room temperature. Make sure the sample mixes with the resin during incubation period. Alternatively, gently vortex every few minutes.
5. After incubation, snap off bottom closure and loosen the top cap. Place the mini column into a 2 mL collection tube and centrifuge at  $1000 \times g$  for 2 minutes.
6. Discard the column containing the resin.
7. The filtrate contains sample with albumin and IgG removed. Use for further processing or store at  $-20^{\circ}\text{C}$  for later use. The depleted sample will be in 10 mM PBS and 0.02% sodium azide, pH 7.4.

**Note:** Sample processing will depend on the type of downstream analysis and may require buffer exchange, lipid and other metabolite removal and/or concentration for 2D gel electrophoresis or MS analysis. Use Thermo Scientific™ Pierce™ Protein Concentrators for buffer exchanging and/or concentrating.

## Remove HSA/IgG in midi format

1. Equilibrate the depletion spin column to room temperature.
2. Remove the column screw cap and add 100  $\mu$ L of sample directly to the resin slurry in the column.
3. Cap the column and invert the column several times until the resin is homogenous in solution.
4. Incubate the mixture in column with gentle end-over-end mixing for 10 minutes at room temperature. Make sure the sample mixes with the resin during incubation period. Alternatively, gently vortex every few minutes.
5. After incubation, snap off the bottom closure and loosen the top cap. Place the column into a 15 mL collection tube and centrifuge at  $1000 \times g$  for 2 minutes.
6. Discard the column containing resin.
7. Filtrate contains sample with albumin and IgG removed. Use for further processing or store at  $-20^{\circ}\text{C}$  for later use. The depleted sample will be in 10 mM PBS and 0.02% sodium azide, pH 7.4

**Note:** Sample processing will depend on the type of downstream analysis and may require buffer exchange lipid and other metabolite removal and/or concentration for 2D gel electrophoresis and MS Analysis. Use Thermo Scientific Pierce Protein Concentrators for buffer exchanging and/or concentration (see the Related products section).

## Troubleshooting

Observation	Possible cause	Recommended action
Albumin or IgG were not completely removed.	Sample exceeded binding capacity.	Reduce amount of sample processed.
	Incomplete binding.	Increase incubation time.
	Sample was not mixed during incubation.	Mix the sample with resin with gentle end-over-end mixing and make sure that the sample is mixing with the resin during the incubation period.

## Limited product warranty

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