



Human Serum Albumin (HSA) Depletion Column (IgY kit)

Catalog Number: GWB-HSAIGY
Product Form: Bead slurry in TBS

Product Description:

Catalog #	Description	Observed Capacity*
GWB-HSAIGY	2 depletion spin columns, each pre-packed with 0.5ml anti-HSA IgY beads	30 - 40µl human serum or plasma for each spin column At least 20 cycles for each spin column

Affinity-purified anti-HSA IgY antibody covalently conjugated through its Fc portion.

*The column is reusable for at least 20 times under proper conditions

Antibody Source: Chicken polyclonal. Antigen affinity purified

Buffer: Tris-Buffered Saline (10mM Tris-HCl, 150mM NaCl, pH 7.4) with 0.02% sodium azide.

Storage Instructions: 4°C. **Do not freeze.**

Application: Plasma or serum protein separation, immunoprecipitation.



PROTOCOL

Materials Provided

1. Two pre-packed 0.5 ml beads anti-HSA IgY gel spin columns (Bio-Rad Micro Bio-Spin Columns -Cat. No. 732- 6204)
2. Six Spin Column Ends (Bio-Rad Cat. No. 731-1660)

Materials Not Provided

1. 2ml collection tubes
2. Buffers:
 - a. Dilution Buffer: Tris Buffered Saline (10 mM Tris, 150 mM NaCl, pH 7.4) (30ml)
 - b. Wash Buffer: Dilution Buffer containing 0.05% Tween-20 (30ml)
 - c. Stripping Buffer: 0.1M Glycine, pH 2.5 (30ml)
 - d. Neutralization Buffer: 1M Tris-HCl, pH 8.0 (10ml)

Procedure for one pre-packed spin column

Immunocapture of HSA

1. Dilute 30 or 40 μ l serum or plasma sample in 270 or 360 μ l TBS to get a final volume of 300 or 400 μ l respectively.
2. Invert the spin column several times to re-suspend the beads, snap off the tip from the column and place the column in a 2ml microcentrifuge tube. Discard the flow-through.
3. Centrifuge the column for 1 minute at 500 xg in a microcentrifuge to obtain dried beads.
4. Place the end cap to the column tip. Add 0.5ml of TBS to equilibrate the beads. Seal the column with the top snap cap. Invert the column several times to mix the beads.
5. Remove the end cap and place the column in a 2ml microcentrifuge tube. Centrifuge for 1 minute at 500 xg. Discard the flow-through.
6. Place the end cap to the column tip. Immediately add 0.3 or 0.4ml diluted serum or plasma sample to the dried beads in the column. Seal the column with the top snap cap.
7. Invert the column several times to mix the beads and the sample, place it on an end-to-end rotator and incubate at room temperature for 15 minutes.
8. Invert the column. Remove the end cap and place the column in a 2ml microcentrifuge tube. Centrifuge for 1 minute at 500 xg. Collect flow-through (HSA-depleted) sample for

further analysis.

9. To obtain maximum yields of flow-through samples, an optional wash step can be applied. Add 0.5ml TBS to the beads. Invert the column several times to mix beads and buffer. Centrifuge for 1 minute at 500 xg. Collect and combine with the flow-through sample from step 8 for further analysis. Samples can be further concentrated using an Amicon Ultra centrifugal filter device (Millipore, Catalog # UFC501096) to desired concentration and volume.

Stripping of Bound Protein

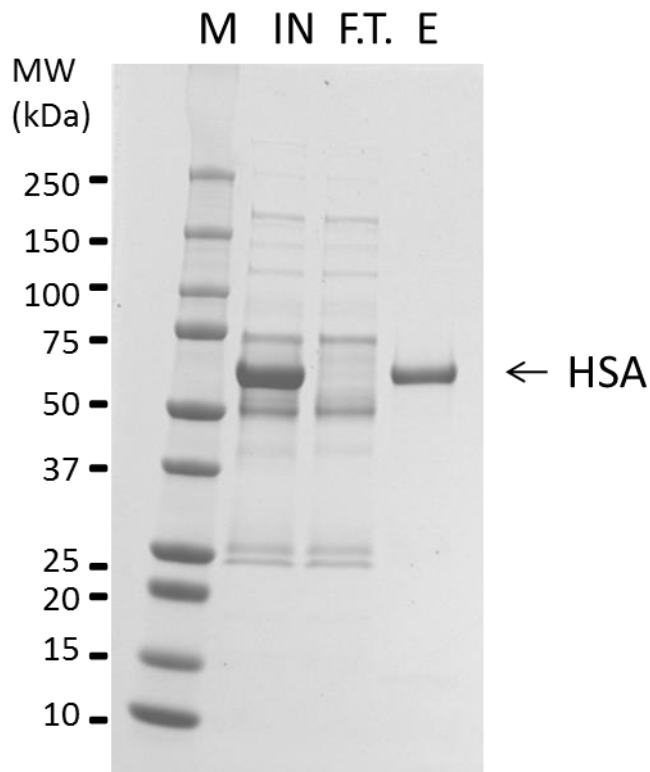
1. To remove proteins non-specifically bound to beads, wash beads with 0.5ml of TBS containing 0.05% Tween-20 a total of 3 times. For each wash, always first insert the end cap, and then add 0.5ml washing buffer and seal the column with top snap cap. Invert the column several times to mix the beads and buffer, remove the end cap while inverting the column and place it in a 2ml microcentrifuge tube. Centrifuge for 1 minute at 500 xg and save the flow-through for further analysis.
2. Strip off bound proteins from beads using 0.5ml of Stripping Buffer (0.1M Glycine-HCl, pH 2.5), a total of 2 times. For each elution, place the end cap to the column first after centrifugation, then add 0.5ml Stripping Buffer and seal the column with top snap cap. Invert the column several times to mix the beads and buffer, incubate at room temperature for 2-3 minutes, remove the end cap while holding the column upside down and place it in a 2ml microcentrifuge tube. Centrifuge for 1 minute at 500 xg and collect the eluent. **It is crucial for column stability to immediately neutralize the beads** (see section **Regeneration of anti-HSA IgY beads**).
3. Pool two eluted samples (total ~1ml) and neutralize with 100µl of Neutralization Buffer. Samples can be further concentrated using an Amicon Ultra centrifugal filter device (Millipore, Catalog # UFC501096) to desired concentration and volume.

Regeneration of anti-HSA IgY beads

1. Add 0.5ml of Stripping Buffer and wash the column a total of **2 times**. For each wash, place the end cap to the column first after centrifugation, then add 0.5ml Stripping Buffer and seal the column with top snap cap. Invert the column several times to mix the beads and buffer, incubate at room temperature for 2-3 minutes, remove the end cap while holding the column upside down and place it in a 2ml microcentrifuge tube. Centrifuge for 1 minute at 500 xg and discard the flow through.

2. After stripping, **immediately** neutralize beads with 0.5ml of 1:10 diluted Neutralization Buffer (100 mM Tris-HCl, pH 8.0). Invert the column several times or briefly vortex to mix beads and buffer. Incubate at room temperature for 5 minutes.
3. Spin down beads in the column for 60 seconds at 500 xg.
4. Re-suspend beads in 0.5ml TBS. Beads are ready for storage at 4°C or next separation. For storage of regenerated beads, it is suggested that the storage buffer TBS contain 0.02% sodium azide (NaN₃).

Depletion Assay for HSA



M: MW marker, IN: Input, F.T: Flow Through depleted for HSA, E: Elution bound proteins from anti-HSA beads

4-20% SDS PAGE under reducing conditions.