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IgG (Fc) Depletion Column (IgY Kit)

Catalog Number: GWB-IGGIGY

Product Form: 50% bead slurry in TBS

Product Description:

Catalog #	Description	Observed Capacity*
GWB-IGGIGY	2 spin columns, each pre-packed with 0.5ml anti-IGG IgY beads	60-80μl human serum or plasma for each spin column At least 20 cycles for each spin column

Affinity-purified anti-IgG IgY antibody covalently conjugated through its Fc portion to 60μm polymeric beads.
*The gels are reusable for at least 20 times under proper conditions

Antibody Source: Chicken polyclonal. Antigen affinity purified

Cross Reactivity: The product may be used to capture rat and mouse IgG though capacity may be reduced for non-human samples.

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.

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PROTOCOL

Materials Provided

1. Two pre-packed 0.5 ml (50% slurry beads) anti-IGG 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

Note: Before using the column 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.

Immunocapture of IgG

1. Dilute 60-80μl serum sample in 440-420μl TBS to get a final volume of 500μl.
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.
3. Centrifuge the column for 60 seconds at 500 xg in a microcentrifuge to obtain dried beads.
4. Place the end cap to the column tip. Immediately add 0.5ml diluted serum sample to the dried beads in the column. Seal the column with the top snap cap.
5. Invert the column several times or briefly vortex the column to mix the beads and the sample, place it on an end-to-end rotator and incubate at room temperature for 15 minutes.
6. Invert the column. Remove the end cap and place the column in a 2ml microcentrifuge tube. Centrifuge for 60 seconds at 500 xg. Collect flow-through (IgG-depleted) sample for further analysis.
7. 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 or briefly vortex to mix beads and buffer. Centrifuge for 60 seconds at 500 xg. Collect and combine with the flow-through sample

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from step 6 for further analysis. Samples can be further concentrated using a Microcon YM-10 centrifugal filter device (Amicon, Catalog # 42421) 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 or briefly vortex to mix the beads and buffer, remove the end cap while inverting the column and place it in a 2ml microcentrifuge tube. Centrifuge for 60 seconds 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 or briefly vortex 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 60 seconds at 500 xg and collect the eluent. **It is crucial for column stability to immediately neutralize the beads** (see section **Regeneration of anti-IgG IgY beads**).
3. Pool two eluted samples (total ~1ml) and neutralize with 100µl of Neutralization Buffer. Samples can be further concentrated using a Microcon YM-10 centrifugal filter device (Amicon, Catalog # 42421) to desired concentration and volume.

Regeneration of anti-IgG 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 or briefly vortex 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.

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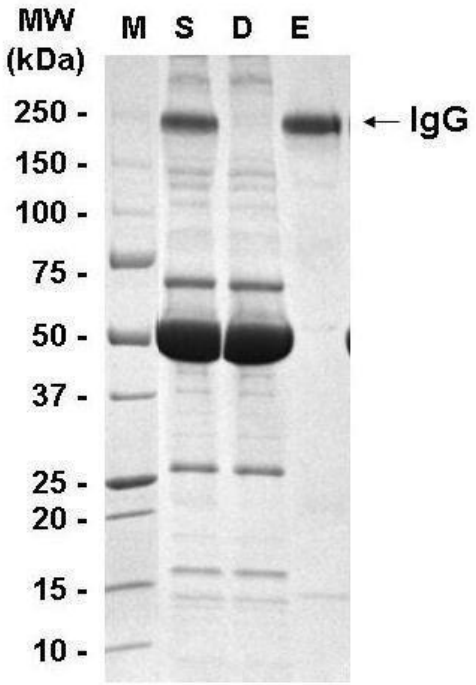
2. After stripping, **immediately** neutralize beads with 0.6ml of 1:10 diluted Neutralization Buffer (100 mM Tris-HCl, pH 8.0). Invert the column several times or briefly vortex to mix beads and buffer. Incubate at room temperature for 5 minutes.
3. Spin down beads in the column for 60 seconds at 500 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 (NaN_3).

Affinity Separation of IgG from Human Serum or Plasma



Affinity separation of IgG from Human serum

M: Molecular weight marker

S: Human serum pre-depletion

D: IgG-depleted human serum

E: Eluted bound proteins from anti-IgGFc IgY beads.

4 – 20% gradient SDS-PAGE, non-reducing condition.