GenWay Biotech, Inc.
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Product Datasheet

Pre-packed Affinity-Purified Anti-Human Fibrinogen IgY Gel Kit

Catalog number: GWB-HFBIGY

Product Form: 50% bead slurry in TBS

Product Description:

Catalog #	Description	Observed Capacity*
GWB-HFBIGY	1.0ml anti-Fibrinogen IgY gel pre-packed in a spin column	125-150μl human serum or plasma
	Dilution Buffer (30ml) Wash Buffer (30ml) Stripping Buffer (30ml) Neutralization Buffer (10ml)	At least 15 cycles

Affinity-purified anti-Fibrinogen 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 Produced From:

Chicken, polyclonal. Antigen affinity-purified.

Cross Reactivity:

The product may be used to capture rat and mouse Fibrinogen, though capacity may be reduced for non-human samples. Contact GenWay for specific information.

Buffer:

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

Storage Instruction:

4ºC. **Do not freeze**.

Recommended Application:

Plasma or serum protein separation, immunoprecipitation.

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Batch Protocol for Immunocapture of Fibrinogen Using a Pre-packed Spin Column

Materials

1. Pre-packed anti-Fibrinogen IgY gel in a spin column (Bio-Rad Micro Bio-Spin Columns -Cat. No. 732-6204)

2. Buffers:

- Dilution Buffer: Tris Buffered Saline (TBS, 30ml)
- Wash Buffer: Dilution Buffer containing 0.05% Tween-20 (30ml)
- Stripping Buffer: 0.1M Glycine, pH 2.5 (30ml)
- Neutralization Buffer: 1M Tris-HCl, pH 8.0 (10ml)

Materials Not Provided

1. 2mL Collection Tubes
2. Additonal Spin Column Ends and Sanp Caps (Bio-Rad Cat. No. 731-1660).

Methods

Immunocapture of Fibrinogen

1. Dilute 125µl serum sample in 500µl TBS to get a final volume of 625µl (1:5 dilution).
2. Invert the spin column several times to resuspend the beads, snap off the tip from the column and place the column in a 2ml microcentrifuge tube
3. Centrifuge the column for 15 sec at 5,000 rpm 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 min.
6. Invert the column. Remove the end cap and place the column in a 2ml microcentrifuge tube. Centrifuge for 15 seconds at 5,000 rpm. Collect flow-through (Fibrinogen-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 15 seconds at 5,000 rpm. Collect and combine with the flow-through sample from step 6 for further analysis.

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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 15 seconds at 5,000 rpm 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 min, remove the end cap while holding the column upside down and place it in a 2ml microcentrifuge tube. Centrifuge for 15 seconds at 5,000 rpm and collect the eluent. **It is crucial for column stability to immediately neutralize the beads** (see section **Regeneration of anti-Fibrinogen 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-Fibrinogen IgY beads

1. To regenerate beads after stripping bound serum proteins as described above, **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 min.
2. Spin down beads in the column for 15 seconds at 5,000 rpm.
3. Resuspend beads in 0.5ml TBS. Beads are ready for storage at 4^{o}C or next separation. For storage of regenerated beads, it is suggested that the storage buffer TBS contain 0.02% sodium azide (NaN_3).

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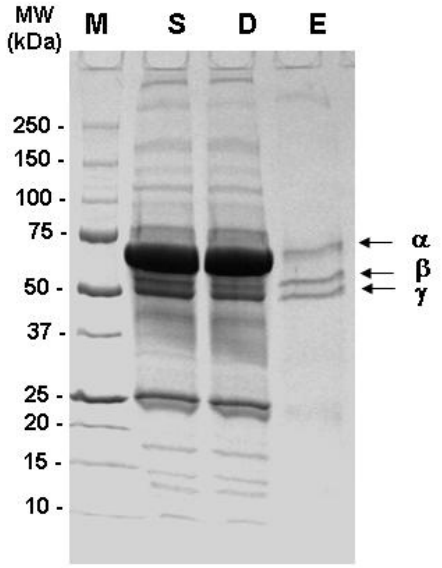
Removal of Fibrinogen from Human Plasma



M: MW marker

S: 1:10 dilution human plasma before depletion

D: S after depletion with anti-Fibrinogen IgY beads

E: Eluted bound proteins from anti-Fibrinogen IgY beads

α, β, γ: Fibrinogen fragments