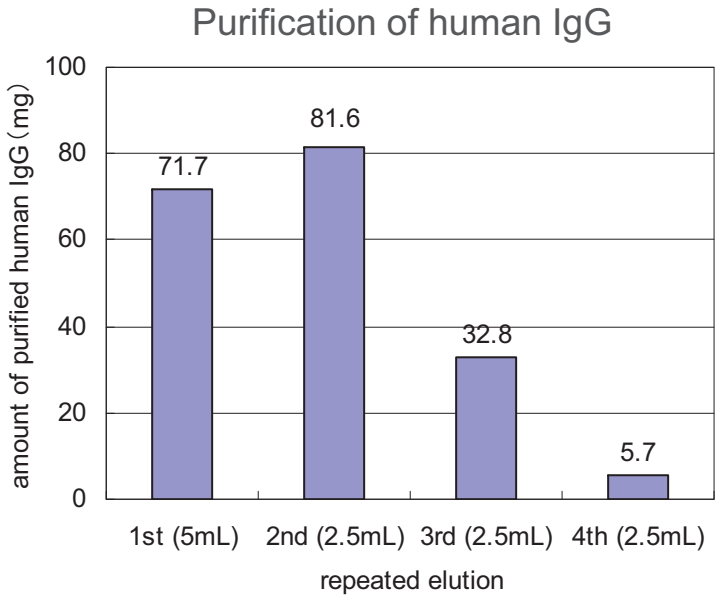


A solution of human IgG solution (30mg/mL, 8 mL) was applied to the column and IgG was purified according to “Standard Protocol”. Total 191.8 mg of IgG was purified with 4-times elution. 1st fraction eluted with 2 bed volumes (5 mL) contained 37% of total purified IgG. When 2nd fraction eluted with 1 bed volume (2.5 mL) was added to 1st fraction, it was increased to 79.9%. When 3rd fraction eluted with 1 bed volume (2.5 mL) was added to the mixture of 1st and 2nd fractions, 97.7% of total purified IgG was recovered.

Order Information

Product Name	Contents	Code No.
Ab-Capcher ExTra	2 mL 10 mL 100 mL	P-003-2 P-003-10 P-003-100
Ab-Rapid SPiN EX (Spin column)	0.1 mL gel/column x 10 (20 empty 2-mL tubes included)	P-014-10
	2.5 mL gel x 2, empty column x 2	P-014-5-1
Buffer Kit	1 kit (Bind. 200mL, Elut. 30mL, Neutr. 1mL)	P-011

There are cases that prices will be changed without notice.
For research use only.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO EKIMAE BLDG. 2-20, TOYO 2CHOME
KOTO-KU, TOKYO 135-0016, JAPAN
TEL : +81-3-5632-9617
FAX : +81-3-5632-9618

ProteNova Co.Ltd. Takamatsu Lab.
2217-44 Hayashi-cho, Takamatsu
Kagawa 761-0301 Japan
TEL +81-87-897-2073 FAX +81-87-816-2073
URL http://protenova.com

ProteNova
INNOVATION BY PROTEIN ENGINEERING

Ab-Rapid SPiN EX-5mL

P-014-5-1

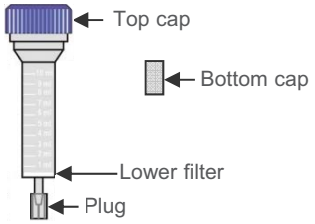
Ver.1.2

Ab-Rapid SPiN EX 5mL specifications

- Gel volume: Ab-Capcher ExTra, 2.5mL x 2 (50% gel slurry, 5mL x 2)
- Spin column: 2 (Max. 22 mL / column)
- Top cap: 2
- Bottom cap: 2
- Gel matrix: Highly closslinked agarose
- Particle size: approx. 35μm
- Ligand: Alkali-resistant Protein A derivative (Protein A-R28)
- Binding capacity (saturated): approx. 190 mg human IgG / column
- Form: 20% Ethanol
- Storage: 4-8℃

How to use of Snap-Off plug

Before use of the column, snap-off the plug and use the bottom cap.



Materials

- Centrifuge (swing or angle type, < 3,000 rpm, the column can be used at 3,000 × g)
- 50mL conical tube
- Buffers
  - Binding Buffer: PBS
  - Elution Buffer: 0.1 M Glycine-HCl, pH 2.5 - pH 3.0
  - Neutralization Buffer: 1 M Tris

＊Buffer Kit (Set of buffers needed for antibody purification) is on sale. (See Order Information)

Sample preparation (example)

◆ Ascites : 3 x dilution with Binding Buffer.
◆Serum: precipitation with 50%-saturated $(NH_4)_2SO_4$ or 5 x dilution with Binding Buffer
◆Cultured medium: Adjust pH to neutral.

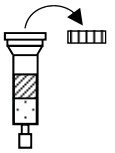
Recommended pre-treatments of sample before applying to column.
- Centrifugation; 10,000 × *g*, 10 min
- Filtration; 0.45μm filter
  （Please use low-protein-adsorption types）

＊If there are insolubles in the sample, make sure to do pre-treatments.

Preparation for 50% ammonium sulfate precipitation
1. Prepare saturated ammonium sulfate.
   Add equal volume of saturated ammonium sulfate gradually to serum and mix.
2. Stand on ice for more than 1hr.
3. After centrifugation at 4℃, remove the supernatant.
   Wash precipitate with 50%-saturated ammonium sulfate.
4. Resolve the precipitate with small volume of Binding Buffer. The precipitate contains antibody.
5. Exchange to Binding buffer with dialysis or desalting column.

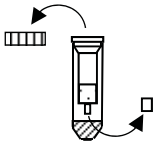
Protocol

Required Time : 2 hrs



Preparation of column and recovery tubes for Elution
- Mix a bottle of Ab-Capcher ExTra 2.5mL and add all of content to an empty column.
- Add Neutralization Buffer to 50 mL conical tubes.
  ・・・1st tube, 100μL; 2nd or later tubes, 50 μL (In case of Elution Buffer at pH 2.8)

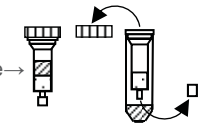
Step 1. Equilibration



- Snap off the outlet plug of the column and set it into a 50mL conical tube.
- Remove preservative solution by centrifugation at 3,000 x rpm for 30 seconds.
- Close the bottom cap, add 7.5 mL of Binding Buffer, agitate the column, set it into the 50 mL conical tube and centrifuge at 3,000 x rpm for 30 seconds.

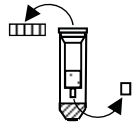
Repeat this step once more.
If buffer remains in the column, centrifuge for longer time.

Step 2. Sample Apply



- Close the bottom cap tightly and add prepared sample to the column.
- Close the top cap and incubate for 1~2 hrs with mixing for the gel not to sink .
- Put off the bottom cap, set the column into a 50 mL conical tube and centrifuge at 3,000 x rpm for 30 seconds.

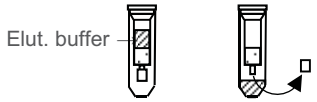
Step 3. Wash



- Put off the top cap, add 10 mL of Binding Buffer, close the bottom cap and shake for 5 min.
- Put off the bottom cap and centrifuge at 3,000 x rpm for 30 seconds.
- Repeat this step more 2 times.

(If non-specific proteins should be reduced, repeat total 5 times)

Step 4. IgG-Elution



- Close the bottom cap tightly and add 5 mL of Elution Buffer.
- Close the top cap and shake for 5 min.
- Put off the bottom cap, set the column into a 50 mL conical tube including Neutralization Buffer and collect eluate in the tube by centrifugation at 3,000 x rpm for 30 seconds.
- For 2nd and 3rd elution, add 2.5 mL of Elution Buffer to the column, shake 5 min, in another 50 mL conical tube including Neutralization Buffer, respectively.

Approx. 37% of purified IgG is collected in 1st eluate and 43% of it is in 2nd eluate.
If higher concentration of IgG is needed, use mixture of 1st and 2nd eluate (approx. 80% recovery).
If higher amount of IgG is needed, use mixture of 1st to 3rd eluate (approx. 97% recovery).

Note
- Spin column (included) is available up to 3,000 x g.
- Centrifuge conditions: Swing rotor (r = 10 cm), at 3,000 rpm (1,000 x g), for 30 sec. (standard protocol) Angle rotor (r = 8.1 cm), at 3,000 rpm (815 x g), for 30 sec.
- Some of angle rotors, if top of the column touch the lid of centrifuge, cut short the conical tube.
- If sample volume is over the tube capacity, discharge of the sample cannot complete at 1st centrifugation. After 1st centrifugation, discard the fluid in the tube and centrifuge again.