Attention (for increase in binding capacity)

"**Standard Protocol**" is suited for purification of "high-binding-capacity" antibodies such as human or mouse IgG. "**Saturation Protocol**" , described below, is recommended for such as rat IgG or "low-binding-capacity" antibodies or first trial of the item.

Saturation Protocol

Note: If IgG-binding is too weak, use 1.5 M Glycine-3.0 M NaCl (pH 9.0) as binding buffer, after confirmation that antibody is not inactivated.

Step 1. Equilibration

・Same as "Standard Protocol".

Step 2. Sample Apply

・Plug into outlet of a column tightly and add prepared sample to the column.
・Close a screw cap and shake the column vigorously for 1-2 hrs to avoid sinking gel.
・Put off the outlet plug, set the column into a 2mL-tube and centrifuge at 2,000 x g for 5 seconds.

Step 3. Wash

・Put off the screw cap, add 0.6 mL of Binding Buffer, plug into column-outlet and shake for 5 min.
・Put off the outlet plug, set the column into a 2 mL-tube and centrifuge at 2,000 x g for 5 seconds.
・Repeat these steps for more 2 times.

Step 4. IgG-Elution

・ Plug into outlet of a column tightly and add 0.2 mL of Elution Buffer.
・ Close a screw cap and shake the column vigorously for 5 min to avoid the gel sinking.
・ Put off the outlet plug, set the column into a 1.5 mL-tube containing Neutralization Buffer. Eluate is collected into the tube after centrifugation at 2,000 x g for 5 seconds.
・ Set the column into another 1.5 mL-tube containing Neutralization Buffer and repeat the same procedure. (If pH of Elution Buffer is higher than 3, repeat once again and collect 3rd eluate.)

Note: ・In some species of antibody, binding to the gel may be weak.
・In some molecular species of Rat IgG2a, binding to the gel may be weak (EX : about 1mg/mL gel)
・In mouse IgM, there are 2 type of molecular species. "High-binding" type can be purified with this protocol, but "low-binding" type is difficult to be purified.

Order Information

Product Name	Contents	Code No.
Ab-Capcher ExTra	2 mL	P-003-2
	10 mL	P-003-10
	100 mL	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
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

Users Manual

P-014-10

Ver.1.2

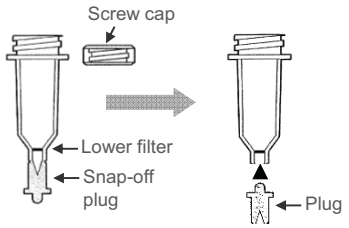
Ab-Rapid SPiN EX Specifications

This column includes endotoxin-tested Ab-Capcher ExTra.

- Gel volume: 0.1 mL (25% gel slurry, 0.4mL)
- Gel matrix: Highly crosslinked-agarose (Rapid Run)
- Column volume: 0.8 mL
- Particle size: 35 µm
- Ligand: Alkali-resistant Protein A derivative (Protein A-R28)
- Binding Capacity: approx. 7.5 mg human IgG /column
- Form: 20% ethanol
- Storage: 4-8℃
- Accessories: 20 empty 2 mL-tubes

How to use Snap-off plug

Snap off the outlet plug and use its reverse side for closing



Materials

- bench-top centrifuge (1,000 - 2,000 × g)
- 1.5mL micro-centrifuge 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 available. (See Order Information)

Sample preparation (example)

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

Recommended pre-treatments of sample before applying to the 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.

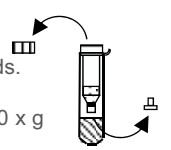
Preparation for 50% ammonium sulfate precipitation
1. Prepare saturated ammonium sulfate.
   Add equal volume of saturated ammonium sulfate gradually to serum and mix.
2. Stand on ice for more than 1hr.
3. After centrifugation at 4℃, remove the supernatant.
   Wash precipitate with 50%-saturated ammonium 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.

"Standard Protocol" — Required Time : 10 min

Preparation of tubes for Elution
Add Neutralization Buffer to 1.5mL micro-centrifuge tubes.
Elution Buffer(pH 2.5)・・・ 1st tube, 5 µL; 2nd tube, 9 µL
Elution Buffer(pH 2.8)・・・ 1st tube, 4 µL; 2nd tube, 5 µL

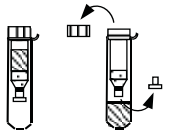
Step 1. Equilibration — 1 min

- If gel attached to top of column, mix up-side-down several times.
- Put off an outlet plug and set a column into a 2mL-tube (included).
- Remove preservative solution by centrifugation at 2,000 x g for 5 seconds. (If lid prevent centrifuge, cut off lids of tubes before centrifugation.)
- Put off a screw cap, add 0.6 mL of Binding Buffer and centrifuge at 2,000 x g for 5 seconds. If buffer remains in the column, centrifuge for longer time.



Step 2. Sample Apply — 4 min

- Plug into outlet of the column tightly and add prepared sample to the column.
- Close the screw cap and incubate for 4 min with mixing every 30-60 seconds.
- Put off the outlet plug, set the column into a 2 mL-tube and centrifuge at 2,000 x g for 5 seconds.



Step 3. Wash — 1 min

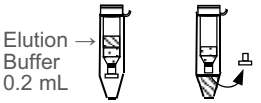
- Put off the screw cap, add 0.6 mL of Binding Buffer so that the gel is suspended, and centrifuge at 2,000 x g for 5 seconds.
- Repeat this step more 2 times in bench-top centrifuge.

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

Step 4. IgG-Elution — 1 min × 2

- Plug into outlet of the column tightly and add 0.2 mL of Elution Buffer.
- Close the screw cap, mix by tapping and leave to stand for 1 min.
- Put off the outlet plug, set the column into a 1.5 mL-tube including Neutralization Buffer and collect eluate in the tube by centrifugation at 2,000 x g for 5 seconds.
- Repeat the same steps, collect 2nd eluate in another 1.5 mL-tube including Neutralization Buffer.

(80% of purified IgG is collected in 1st eluate and 20% of it is in 2nd eluate. If higher concentration of IgG is needed, use 1st eluate. Mixture of 1st and 2nd eluate is also available.)



〈 For increase in binding capacity 〉

See Next Page

Required Time : 2 hrs

"Saturation Protocol"

- Incubation time of sample and gel in Step 2 is changed from 4 min to 1-2 hrs.
- In Washing and Elution (Step 3 and 4), shake for 5 min before all centrifugation steps.