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 binding to antibody 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 into 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 2 seconds.

Step 3. Wash

- Put off the screw cap, add 0.6 mL of Binding Buffer, plug into outlet of the column 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 2 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 sinking gel.
- 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 2 seconds.
- Set the column into another 1.5 mL tube containing Neutralization Buffer and repeat the same procedure. (If Elution Buffer is upper than pH3, repeat once again and collect 3rd eluate.)

Note: - In some species of antibody, binding to antibody may be weak.
- In some molecular species of Rat IgG2a, binding to antibody 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-Rapid SPiN 10	0.1mL x 10 columns	P-013-10
・Ab-Rapid SPiN 50	5 mL gel x1 bottle, empty columns x 50	P-013-50
・Buffer Kit	Bind. Buf. 200 mL, Elut. Buf. 30 mL, Neutr. Buf. 1mL	P-011

Related products

Product name	Contents	Code No.
・Ab-Capcher	2 mL gel x1 bottle	P-002-2
	10 mL gel x1 bottle	P-002-10
・Ab-Rapid PuRe 2	Column x 2, 2.5 mL syringe x 1	P-012-2
・Ab-Rapid PuRe 10	Column x 10, 2.5 mL syringe x 1	P-012-10

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ProteNova
INNOVATION BY PROTEIN ENGINEERING

Ab-Rapid SPiN™

Users Manual

P-013-10

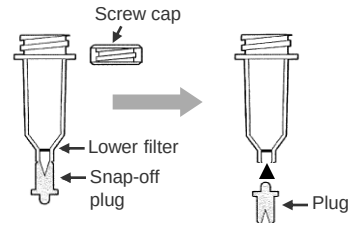
Ver.3.3

Ab-Rapid SPiN Specifications

- Gel volume: 0.1 mL (25% gel slurry, 0.4mL)
- Gel matrix: Highly-crosslinked-agarose
- Column volume: 0.8 mL
- Particle size: 45-165 µm
- Ligand: Alkali-resistant Protein A derivative (Protein A-R28)
- Binding Capacity: approx. 4.5 mg human IgG /column
- Storage: 20% ethanol
- Accessories: 2 mL empty tube x 20

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 on sale. (See Order Information)

Sample preparation (example)

◆ Ascites : 3 x dilution with Binding Buffer.
◆Serum: Ppt. 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.

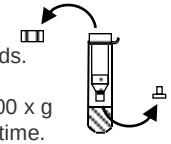
Preparation for 50% ammonium sulfate precipitation
1. Prepare saturated ammonium sulfate.
   Add equal volume of saturated ammonium sulfate gradually to serum and mix.
2. Stand on ice for more than 1hr.
3. After centrifugation at 4℃, remove the supernatant.
   Wash precipitate with 50%-saturated ammonium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

"Standard Protocol" — Required Time : 10 min

Preparation of tubes for Elution
Add Neutralization Buffer into 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

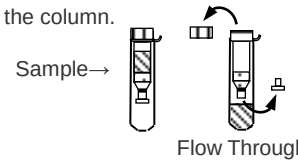
Step 1. Equilibration — 1 min

- If gel attached to top of column, mix up-side-down several times.
- Put off an outlet plug and set a column into 2mL tube (included).
- Remove preservative solution by centrifugation at 2,000 x g for 2 seconds. (If lids 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 2 seconds. If buffer remains in the column, centrifuge for more long time.



Step 2. Sample Apply — 4 min

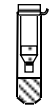
- Plug into outlet of the column tightly and add prepared sample into the column.
- Close the screw cap and incubate for 4 min with mixing every 30-60 seconds.
- Put off the outlet plug, set the column into a 2 mL tube and centrifuge at 2,000 x g for 2 seconds.



Step 3. Wash — 1 min

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

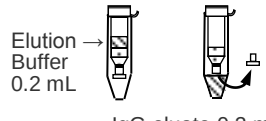
(If non-specific proteins should be reduced, repeat total 5 times)



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 2 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.