Technical Information

Protein A-R28 is an alkali-tolerant IgG-binding protein derived from protein A, which is developed with ProteNova's patent technology. Protein A-R28 strongly binds to various species and subclasses of IgG, compared with Protein A and G. The coupling to resin (Ab-Capcher) provides an alkali-washable unique affinity medium with high binding capacity for immunoglobulin, which is useful for purification of human, rabbit, mouse and rat IgGs. Ab-Capcher is also useful for immuno-precipitation experiments.

Table 1. Binding properties of Ab-Capcher (Ab-Rapid PuRe)

Species	Sub class	Ab-Cap	Protein A	Protein G
Mouse	IgG1	++++	+	++
	IgG2a	+++++	++++	+
Rat	IgG1	++++	-	+
	IgG2a	+++	-	+
Goat	IgGs	++++	-	+
Chicken	IgY	-	-	-
Human	IgG	+++++	++++	++
Rabbit	IgG	+++++	++++	++

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 PuRe EX 2	Column x 2, 2.5 mL syringe x 1	P-015-2
Ab-Rapid PuRe EX 10	Column x 10	P-015-10
Ab-Rapid SPiN EX (Spin column)	0.1 mL gel/column x 10 (20 empty 2 mL-tubes included)	P-014-10

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

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INNOVATION BY PROTEIN ENGINEERING

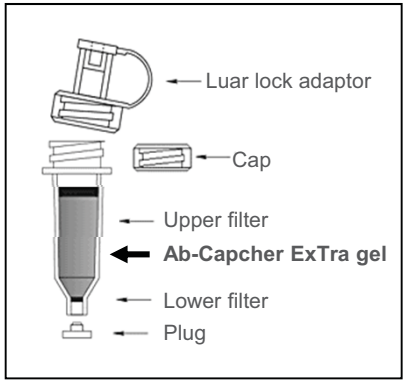
Ab-Rapid PuRe EX

Users Manual
P-015-2
Ver.1.4

Ab-Rapid PuRe Specifications

"Endotoxin-tested " Ab-Capcher ExTra is prepacked.

- Gel volume: 0.5 mL
- 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: >45 mg human IgG /column
- Form: 20% ethanol
- Storage: 4-8℃



Materials

- 2.5 mL syringe
- Microcentrifuge tube
- Buffers
  Binding Buffer: PBS
  Elution Buffer: 0.1 M Glycine-HCl, pH 2.8
  Nuetralization Buffer: 1 M Tris

＊Buffer Kit (PN-011) is also available from ProteNova.
Buffer kit contains Binding Buffer, Elution Buffer and Nuetralization Buffer.

＊If air bubble is present in the space between gel and column
Before use, pass through 10 mL of Binding Buffer into the column by using 10 mL syringe (flow-rate approx. 5mL/min). It is important to press the gel bed. Repeat this procedure until the bubble disappears.

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.

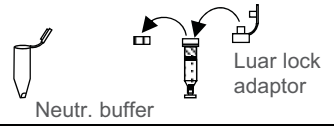
Preparation for 50% ammonium sulfate precipitation
1. Prepare saturated ammonium sulfate.
   Add equal volume of saturated ammonium sulfate gradually to serum and mix.
2. Stand on ice for more than 1hr.
3. After centrifugation at 4℃, remove the supernatant.
   Wash precipitate with 50%-saturated ammonium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

Protocol for IgG Purification

Column pressure is higher and flow rate is slower than those of Ab-Rapid PuRe.

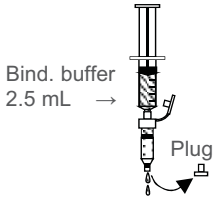
Preparation
Add Neutralization Buffer to a microcentrifuge tube.
(1/30 volume of eluate; 30-35μL to 1 mL of eluate)

Remove a cap on the top of column and fit a luar lock adaptor.



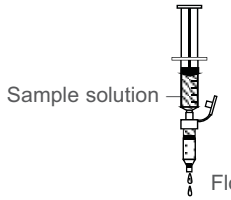
Step 1. Equilibration of Column — 1 min

Fill a syringe with 2.5 mL of Binding Buffer and connect to the top of column.
After removal of a seal and a plug from the bottom of column, pass through 2.5 mL of Binding Buffer at a flow rate of 2.5 mL/min.
Then, remove the syringe.



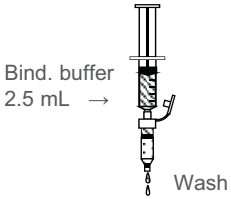
Step 2. Sample Apply to Column — 5 min (for 10 mL)

Apply the sample solution to column using the syringe at a flow rate of 2 mL/min.
Then, remove the syringe.



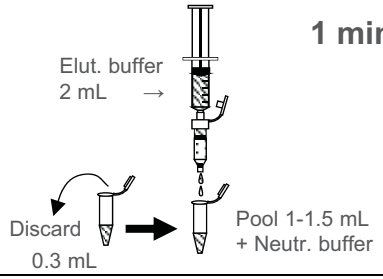
Step 3. Washing of Column — 1 min

Wash the column with 2.5 mL of Binding Buffer with the syringe at a flow rate of 2.5 ml/min.
＊If serum was directly applied to the column, washing with more than 5 mL is recommended.
Then, remove the syringe.



Step 4. Elution of IgG — 1 min

Elute with 2 mL of Elution Buffer using the syringe at a flow rate of 2 mL/min.
For this, discard first 0.3-0.5 mL of eluate and collect the following 1.0 – 1.5 mL of eluate as IgG fraction into a microcentrifuge tube, in which Neutralization Buffer (1/30 volume of eluate) is pre-added, and mix.



Time: within 10 min

＊Storage and reuse of column
- Ab-Rapid PuRe EX column is alkali-washable. After 10 times washing ({5 min x 3} x 10 times), it is confirmed that remained binding capacity is 95% with 0.5N NaOH and 88% with 1.0N NaOH.
- When the column is reused, wash the column with 2.5 mL of 0.1-0.5 N NaOH by syringe after elution of IgG. Using immediately after washing, equilibrate with 2.5 mL of Binding Buffer twice. Then, apply the sample.
- For storage of column, add 2.5 mL of 20% EtOH, tightly close a cap and a plug, and store at 4-8 ℃.