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

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

Order Information

Product name	Contents	Code No.
・Ab-Capcher	2 mL	P-002-2
	10 mL	P-002-10

Related products

Product name	Contents	Code No.
・Ab-Rapid SPiN 10	0.1mL spin column x 10	P-013-10
・Ab-Rapid SPiN 50	5 mL gel x 1, empty spin column x 50	P-013-50
・Ab-Rapid PuRe 2	Column x 2, 2.5 mL syringe x 1	P-012-2
・Ab-Rapid PuRe 10	Column x 10	P-012-10
・Buffer Kit	Bind. Buf. 200 mL, Elut. Buf. 30 mL, Neutr. Buf. 1mL	P-011

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ProteNova
INNOVATION BY PROTEIN ENGINEERING

Ab-Capcher™

Users Manual

Ab-Capcher Specifications

- Gel matrix: 4% cross-linked agarose (Sepharose 4 Fast Flow)
- Particle size: 45-165 µm
- Ligand: Alkali-resistant Protein A–derivatives (Protein A-R28) (*E.coli*)
- Binding Capacity: >65 mg human IgG /mL gel
- Storage: 20% Ethanol at 4-8 ℃

Recommended Reagents

- Buffers
  Binding Buffer: PBS
  Elution Buffer: 0.1 M Glycine-HCl, pH 2.8
  Neutralization Buffer: 1 M Tris

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

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 gel.
- 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

Preparation

Put Ab-Capcher gel into a column.

Add Neutralization Buffer to an empty microcentrifuge tube.
(1/30 volume of eluate: *e.g.,* 30-35µL to 1 mL of eluate)

Step 1. Equilibration

Equilibrate the column with 5 bed volume of Binding Buffer.

Step 2. Sample Applying

Apply the sample solution to column.
(If the bind of the sample seems to be weak, reduce the flow rate.)
Collect the flow through.

Step 3. Column Washing

Wash the column with 5 bed volume of Binding Buffer.

Step 4. Elution of IgG

Add 4 bed volume of Elution Buffer to the column.

Discard first 0.8 bed volume of eluate and collect the following eluate as IgG fraction into a microcentrifuge tube, in which Neutralization Buffer (1/30 volume of eluate) is pre-added, and mix.

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.

＊Storage and reuse of gel
・Ab-Capcher is alkali-washable.
・When the gel is reused, wash the column with 3-5 bed volume of 0.1N NaOH after elution of IgG. Using immediately after washing, equilibrate with 10 bed volume of Binding Buffer. Then, apply the sample.
・For storage of column, add 5 bed volume of 20% Ethanol, transfer to a tightly sealed container and store at 4-8℃.