

IgG Purification Kit - A IgG Purification Kit - G

Technical Manual

Technical Manual (in Japanese) is available at <http://www.dojindo.co.jp/manual/ap.html>

General Information

Dojindo IgG Purification Kit - A and IgG Purification Kit - G can be used for isolation and purification of immunoglobulin G(IgG) from various animals within 30 min. These kits include all necessary components for purification of IgG. Since Dojindo IgG Purification Kits employ silica base protein A or G support unlike agarose base protein A or G support, buffer used in each step is completely removed from protein A or G support after centrifugation to obtain highly purified IgG with high yield. An antibody titer is kept during the purification procedure and the recovered IgG performs well in the following enzyme immuno assay. In addition, protein A or G support in these kits can be repeatedly used 10 times purification with same performance.

Kit Contents

- Protein A/G Cartridge tube	1 tube	
- Washing Buffer	13 ml	x 1 bottle
- Elution Buffer	1.8 ml	x 1 tube
- Catching Buffer	1 ml	x 1 tube
- 1.5 ml Microtube	5 tubes	x 2

Capacity

One sample purification: 50 ml of serum or 200 mg of IgG solution

Storage Condition

Store at 0-5 °C. This kit is stable for one year at 0-5°C in the unopened condition.

Required Equipment and Materials

- 200 µl adjustable pipette
- Microcentrifuge
- Microtubes
- Voltex mixer

Precautions

- The gel in Protein A/G Cartridge tube may be attached to the cap and/or to the side wall of the cup. Tap the bottom of cartridge tube several times against a lab bench to drop the gel on the bottom of the cup prior to open the cap.
- This kit includes ten 1.5 ml Microtubes. Use each 1.5 ml Microtube for each elution procedure(Step 9).

General Protocol for IgG Isolation



1 Mix 50 ml of sample solution containing IgG and 50 ml of Washing Buffer in a microtube^{a)}.



2 Add the mixed solution prepared at Step 1 to the cup of Protein A/G Cartridge tube.
Do not close the cap.



3 Rotate the cup with fingers several times to mix the gel^{b)}. Leave the tube at room temperature for 2 min.



4 Close the cap and centrifuge the tube at 8,000 xg for 30 sec.

5 Transfer the filtrate at Step 4 to the cup again and repeat Step 3 and 4^{c)}. Transfer the filtrate to a microtube^{a)}, and keep it at 0-5 °C^{d)}.



6 Add 200 ml of Washing Buffer to the cup. *Do not close the cap.* Rotate it with a finger several times to mix the gel^{b)}.



7 Close the cap and centrifuge at 8,000 xg for 30 sec. Discard the filtrate of the reservoir.

8 Repeat step 6 and 7.



9 Add 60 ml of Catching Buffer to a 1.5 ml Microtube included in this kit. Put the cup of Protein A/G cartridge tube in this tube^{e)}.



10 Add 70 ml of Elution Buffer to the cup. *Do not close the cap.* Rotate the cup several times with a finger to mix the gel^{b)}.



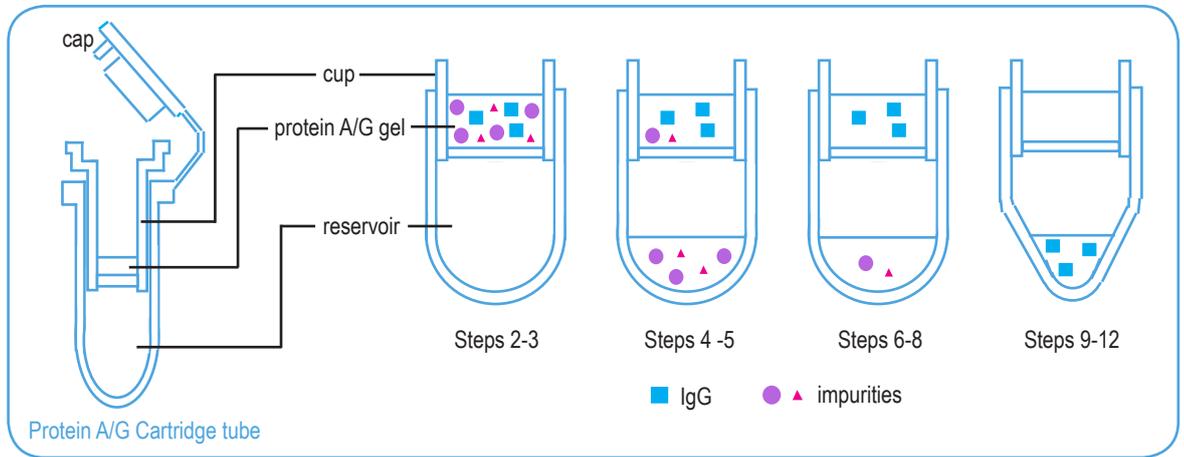
11 Close the cap and centrifuge at 8,000 xg for 30 sec. Keep the filtrate in the microtube.

12 Repeat step 10 and 11.



13 Remove the cup^{f)}. Close the cap and voltex to mix. Store the solution at 0-5 °C^{g)}.

- a) The microtube is not included in this kit. You can use any commercially available microtube.
- b) Slant the cartridge tube and rotate the cup with a finger 10-20 times.
- c) The recovery of IgG increases 25 to 30% more by this process. You can repeat this process to increase a recovery of IgG.
- d) Keep the filtrate for rerecovery. If the recovery of the IgG is low, use this filtrate to recover IgG. Follow the protocol starting at Step 2.
- e) Do not discard the reservoir of Protein A/G Cartridge tube for another purification.
- f) For recycling the used protein A/G gel, put the cup in which used gel is to the reservoir. Wash gel with Washing Buffer by following Step 6 and 7. Add 200 ml of Washing Buffer and keep Protein A/G Cartridge tube at 0-5°C. Centrifuge the cartridge tube to remove Washing Buffer prior to use.
- g) For longer storage, you may add equal volume of glycerol and store at -20°C.



SDS-PAGE of purified IgG and the recovery of IgG from serum 50 ml

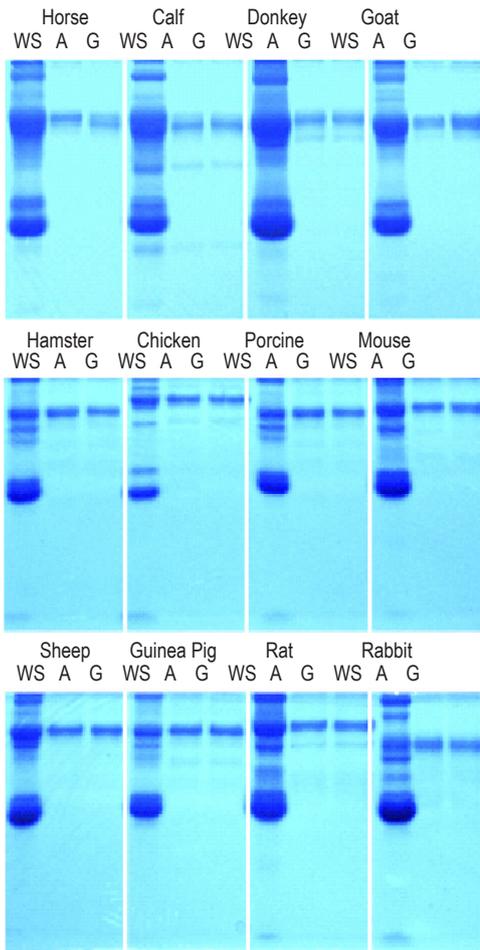


Table. The recovery of IgG from serum 50 ml

	IgG Purification Kit - A (mg)	IgG Purification Kit - G (mg)
Horse	150 - 250	200 - 300
Calf	200 - 300	250 - 350
Donkey	200 - 300	150 - 250
Goat	50 - 100	150 - 250
Hamster	150 - 250	100 - 150
Chicken	25 - 50	10 - 20
Porcine	200 - 300	150 - 250
Mouse	150 - 250	150 - 250
Sheep	50 - 100	150 - 250
Guinea Pig	150 - 200	100 - 200
Rat	50 - 100	100 - 200
Rabbit	200 - 300	150 - 250
Human	150 - 250	200 - 300
Cat	150 - 250	100 - 200
Dog	200 - 300	100 - 200

The amounts of immunoglobulin were determined by reading absorbances at 280 nm.

Figure. SDS-PAGE of purified IgG by using IgG Purification Kit - A and IgG Purification Kit - G

WS : whole serum
A : prepared by IgG Purification Kit - A
G : prepared by IgG Purification Kit - G
6% Acrylamide gel/ Tris-glycine buffer

Normal sera were purchased from Invitrogen, Biomed, and Sigma.

Frequently Asked Questions

- ◆ How is the purity of the purified IgG using this kit?
As shown SDS-PAGE in figure, highly purified IgG was obtained.
- ◆ How much IgG can be recovered from 50 ml of serum?
About 150-350 mg of IgG is recovered from 50 ml of serum. (see table)
- ◆ Can I use the used protein A/G gel for the purification of different IgG solutions?
Use a new gel for a different sample to avoid contamination.
- ◆ I closed the cap before rotate the cup. How should I do?
Centrifuge the cartridge tube at 8,000 x g for 30 sec. Transfer the filtrate to the cup and follow the Steps.

If you require assistance, please contact Dojindo customer service.

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