Amicon® Pro Affinity Concentration Kit – Protein G

Rapid purification or depletion of selective immunoglobulin species.

Catalog Nos. ACR5000PG, ACK5003PG, ACK5010PG, ACK5030PG, ACK5050PG, ACK5100PG

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Revision A

Introduction

The Fc portion of a variety of immunoglobulins (Ig) is known to bind to several bacterial proteins, including Protein A and G. Produced by Group G *Streptococci*, Protein G is a cell wall protein consisting of a single polypeptide chain (MW 46.7 kDa) with minimal carbohydrate modifications. Native protein G has two IgG binding domains as well as sites for albumin and cell surface binding; in recombinant protein G, the latter two domains have been removed to reduce non-specific binding. Agarose-immobilized Protein A and G matrices have been used extensively in research settings where antibody purification is of critical importance. Although the tertiary structures of these two proteins are quite similar, their amino acid compositions differ greatly, resulting in significantly different binding characteristics. This translates to differences in binding affinity of Igs for either format based upon species or origin and isotype (see Table 1). Specifically, Protein G demonstrates greater affinity for Rat IgGs but is unable to bind human IgM, IgD, or IgA. When purifying from samples that are not well defined in the literature (± in Table 1), we recommend preliminary studies to determine the optimal matrix for maximal recovery.

		Protein A	Protein G			Protein A	Protein G			Protein A	Protein G
Human	IgG_1	++	++	Other species (IgG)	Rabbit	++	++	Mouse	IgG_1	+	+
	IgG_2	++	++		Hamster	+	++		IgG_{2a}	++	++
	IgG_3	-	-		Guinea Pig	++	+		IgG_{2b}	++	++
	IgG_4	++	++		Bovine	+	+		IgG_3	+	++
	IgA	+	-		Sheep	±	+		IgM	±	-
	IgD	+	-		Goat	±	+				
	IgE	+	-		Pig	++	++		IgG	++	++
	IgM	+	-		Chicken	-	±		IgG_1	±	+
									IgG_{2a}	±	++
Human Fragments	Fab	+	+		Fc	+	+		IgG_{2b}	±	+
	F(ab')2	+	+		kappa	-	-		IgG_{2c}	±	+
	scFv	+	-		lambda	-	-	Rat	IgM	±	-

Legend:	++	Strong affinity	±	Requires Evaluation
	+	Moderate/Slight Affinity	-	No Affinity

Table 1. Relative affinity of Protein A and Protein G for various immunoglobulins.

Antibody purification using the Amicon Pro Affinity Concentration Protein G Kit consists of three basic steps (binding, wash, and elution) as well as sample concentration and buffer exchange. The high binding capacity of the Protein G matrix also provides a rapid and scalable means for depletion of Igs from serum or plasma allowing detection and analysis of low abundant proteins present in these samples.

By collapsing the entire purification workflow into one device, the Amicon Pro Affinity Concentration System eliminates the need for multiple sample transfers thereby minimizing protein loss. The device offers assay flexibility; samples can be concentrated at the time of elution or during buffer exchange. The increased size of the feeder tube accommodates a greater range of sample capacities as well as reducing the need for multiple centrifugation steps commonly found with other spin-column purification devices during wash and elution steps. The kit contains sufficient Protein G resin, optimized buffers, and Amicon Pro devices for 12 standard reactions.

Product Information

- The Amicon® Pro Affinity Concentration Kit - Protein G (buffers, resin, devices) was optimized for maximal binding and elution of Ig species. If alternative buffers are to be used with the resin, we recommend prior assay optimization to any impact on the extant and/or specificity of protein binding.
- The resin is compatible with commonly used denaturants, detergents and organic solvents.
- Using the Amicon® Pro system, Ig can be purified form serum, ascites fluid, or plasma samples as well as cell culture supernatants. On average, the total IgG content of serum is approximately 10-15 mg/mL irrespective of species. By contrast, concentration of antibody in hybridoma supernatants can demonstrate significant clonal variability. Consider such factors when determining the volume of sample and/or resin to use for a given reaction.
- Antibody fractions purified from cultures grown in the presence of fetal bovine serum (FBS) will also contain Ig present in the FBS.
- For more information, consult the Protein G Agarose User Guide available at http://www.millipore.com/catalogue/item/16-266.

Kit Components

- CS211412- Protein G agarose resin (3 mL) – Crosslinked 4% agarose with immobilized protein G supplied as 50% slurry in distilled H_2O containing 20% ethanol. Protein G is covalently coupled by cyanogen bromide. The binding capacity is typically 20 mg human IgG/mL settled resin.
- Buffers:
  - CS211408-1X Bind/Wash Buffer (2 X 66 mL) – Phosphate Buffered saline (PBS)
  - CS211409-1X Elution Buffer (12 mL) – 0.2 M Glycine pH 2.5
  - CS211410-1X Neutralization Buffer (2.5 mL) – 1M Tris pH 8.5
- Amicon Pro Devices – The kit includes 12 complete assemblies. Each device consists of a exchange device, holder tube, 50 mL collection tube, and Amicon® Ultra-0.5 centrifugal filter device. A 2 mL collection tube is included for sample recovery from the AU-0.5 device by reverse spin. The kit is available in five formats based on the the nominal molecular weight limit (NMWL) of the AU-0.5: 3 (ACK5003PG), 10 (ACK5010PG), 30 (ACK5030PG), 50 (ACK5050PG), and 100kDa (ACK5100PG). Consult the Amicon® Pro (http://www.millipore.com/psp, search keywords "Amicon Pro") and Amicon® Ultra-0.5 centrifugal filter device User Guides (http://www.millipore.com/catalogue/module/c82301) for proper assembly/disassembly and additional product information.

All reagents should be stored at 2 to 8 °C (do not freeze). The Amicon Pro devices can be stored separately at room temperature.

Procedures for using the Amicon® Pro Affinity Concentration Kit – Protein G – Ig purification

The protocol is based on the purification of IgG from 0.5 mL of rabbit serum using 200 µl of Protein G resin (100 µl packed resin). The protocol is linearly scalable for 50-1000 µL of resin slurry. Due to large variability among sample preps, parameters which may require optimization include bead input, binding time, wash, and elution parameters. This protocol includes steps for simultaneous concentration during the elution step as well as buffer exchange using the Amicon® Ultra-0.5 centrifugal filter device.

Note: Given the collection tube's size, it is not necessary to remove the filtrate between the various centrifugation steps. If process samples are to be retained for analytical purposes, the collection tube should be cleared.

Bead Preparation

1. To ensure uniform suspension, vortex Protein G resin thoroughly before adding it to the device.
2. Remove the collection tube cap and open the exchange device cap.
3. Add 200 µL of resin slurry to the base of the exchange device. Close the exchange cap.
   - Up to 500 µl packed resin (1000 µl slurry volume) may be added per device. We recommend using wide-bore tips (Cat. No. 02-707-134, Fisher Scientific) for resin transfer.
4. To remove storage buffer, centrifuge in a swinging bucket rotor at 1000 g X 1 min.
5. Add 500 µL of 1X Bind/Wash Buffer. Centrifuge at 1000 g X 1 min.

Protein Binding

1. Add 500 µL of sample to the exchange device.
   - Up to 9 mL of sample can be added. The volume loaded is determined by the target protein's expression level and resin's binding capacity.
2. Incubate for 60 min at room temp with gentle agitation.
   - We recommend upright agitation on a plate shaker at low setting.
   - End-over-end mixing, particularly with small volumes or for extended time, may result in substantial bead loss to the sides of the feeder tube.
   - The duration of binding time may vary with application.
3. Centrifuge the device at 1000 g X 1 min in a swinging bucket rotor. Recover the sample flow-through from the 50 mL collection tube (optional).
   - To ensure maximal protein capture, collect all resin into solution prior to centrifugation.
4. Add 1.5 mL of Bind/Wash Buffer. Centrifuge at 1000 g X 1 min. Recover the wash fraction from the 50 mL collection tube (optional).
   - Due to the large capacity of the exchange device, the volume of the wash can be increased for greater sample purity. There is no need for multiple wash steps.

Sample Elution

Samples can be eluted without concentration by adding elution buffer and centrifuging (1000g X 2 minute) directly into a clean 50 ml collection tube. Adjust pH of the eluted fraction by adding 75 µL of Neutralization buffer (per 500 µL of Elution buffer). Given the limited volume processing capacity of the AU-0.5 device, we recommend this protocol if elution volumes > 1.5 ml are required.

For simultaneous elution with concentration, attach the Amicon® Ultra-0.5 device and follow the steps outlined below.

1. Remove the exchange device and insert it into the AU0.5 device.
2. Place the exchange device/AU-0.5 assembly back in the holder and return the device to the collection tube.
3. Add up to 1 mL of Elution Buffer, gently resuspend the resin, and incubate for 5 min.
   - Under standard conditions, one elution is sufficient for recovery of 90-95% of captured protein.
4. Close the exchange device cap and screw on the collection tube cap to ensure a proper seal.
5. Centrifuge at 4000g X 10 minutes. This is sufficient spin time to ensure the sample volume has concentrated below the level of the resin bed.
6. Remove the collection tube cap and open the exchange device cap. Add 75 µl Neutralization Buffer to the exchange device. Reseal the caps.
7. Centrifuge for an additional 5 minutes at 4000g.
   - Depending on the starting elution volume, NMWL of AU0.5 device employed, and the degree of concentration desired, the length of the spin time can range for 10-30 minutes. Please consult the **Performance Characteristics** section in the Amicon® Pro Affinity Concentration System User Guide (http://ww.emdmillipore.com/psp, and search keywords "Amicon Pro") for recommended guidelines.
8. Recover the concentrated fraction by reverse spin (see below) or proceed to Buffer Exchange.

Buffer Exchange (**Optional if samples have been collected in the Amicon® Ultra-0.5 device)**

1. After sample concentration, add 1.5ml desired buffer to the exchange device/AU-0.5 assembly.
2. Centrifuge device at 4000g X 15 minutes in a swinging bucket rotor. Concentrated samples can be recovered from the AU-0.5 device by reverse spin (see below).

Collect sample from the AU0.5 device by Reverse Spin (following concentration or buffer exchange)

1. Disassemble the exchange device/AU-0.5 assembly from the holder tube.
2. Using a gentle twisting motion, detach the AU-0.5 from the exchange device.
3. If there is residual sample in the exchange device tip, depress the exchange device cap to expel the remaining sample volume into the AU-0.5.
4. Hold AU-0.5 upright and slide the 2 ml collection tube on top of it.
5. Invert the assembly and centrifuge (in a microcentrifuge) with a fixed angle rotor 1000g X 2min.

Procedures for using the Amicon® Pro Affinity Concentration Kit – Protein G - Ig Depletion from Biological samples

Note: The resin to sample ratio has been increased for the depletion reaction to ensure complete removal of Ig from samples.

Bead Preparation

1. Vortex the Protein G resin thoroughly before adding it to the device.
2. Wash 200 µL Protein G resin slurry in the Amicon® Pro Affinity Concentration device as described previously.

Ig Depletion

Depleted fractions can be collected without concentration by centrifuging (1000g X 2 minute) directly into a clean 50 ml collection tube. Given the limited volume processing capacity of the AU-0.5 device, we recommend this protocol if sample volumes > 1 ml were used.

For simultaneous sample depletion with concentration, attach the Amicon® Ultra-0.5 device to the exchange device and place the assembly in the holder tube in the 50 mL collection tube as previously described. For sample volumes > 1 mL, we recommend sample depletion without simultaneous concentration.

1. Dilute 10-25 µL of sample in 500 µL of Bind/Wash Buffer. Add the sample to the exchange device.
2. Add diluted sample to the base of the exchange device and mix with packed resin by pipeting.
    - Up to 9 ml sample can be added; the volume loaded is determined by the target protein's expression level and/or resin binding capacity.
3. Incubate for 60 minutes at room temperature with gentle agitation (standard plate shaker at low setting).
    - Duration of binding time may vary with application.
    - For larger volume and extended binding reactions, mixing by end-over-end inversion may be preferred. In such cases, we recommend sealing the exchange device cap sealing the exchange device cap with tape over the vent hole (remove tape prior to centrifugation). We do not recommend end-over-end mixing with small volumes; due to insufficient volume, substantial amounts of resin may be lost to the sides of the exchange device.
4. Centrifuge device at 4000g X 15 minutes in a swinging bucket rotor. Concentrated samples can be buffer exchanged or recovered from the AU-0.5 device by reverse spin (described previously).

Wash steps for residual sample recovery

To maximize recovery of proteins in the depleted fraction, under certain conditions, it may be advantageous to wash the resin prior to final sample recovery. Washing should be performed immediately following the depletion step.

1. Add 1.5ml appropriate wash buffer to the exchange device. Place exchange device/AU0.5 assembly back in the holder and return device to the collection tube.
2. Centrifuge device at 4000g for 15 minutes in a swinging bucket rotor. Concentrated samples can be buffer exchanged or recovered from the AU-0.5 device by reverse spin.
    - Depending on the wash volume, NMWL of AU0.5 device employed, and the degree of concentration desired, the length of the spin time can range for 10-30 minutes. Please consult the **Performance Characteristics** section for recommended guidelines.

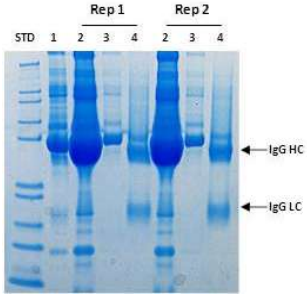
Troubleshooting

Issue: Low Immunoglobulin Binding	
Possible Cause	**Solution**
Insufficient Resin Volume.	Ensure that the resin is well mixed prior to pipeting.
Incorrect resin used.	Refer to Table 1 to match the host and isotype with either Protein A or Protein G resin.
Ig is purified but is degraded - Antibody is sensitive to low pH during elution.	Manipulate pH of elution buffer to preserve Ig function.
Ig is purified but is degraded - Downstream application is sensitive to neutralization buffer.	Perform Buffer Exchange step.
Protein forms aggregates.	Add solubilizing agents such as detergents (0.1% Triton X-100, TWEEN®-20) or increase salt concentration.
Ig elutes in the wash buffer.	Ensure that there are no reducing agents present in the bind/wash buffer.
Protein lost during sample concentration using AU0.5 device.	Check the protein's expected size and MWCO of AU0.5 device used. AU0.5 is offered in 5 different MWCO formats - 3, 10, 30, 50, and 100 kDa.
Protein precipitates during sample concentration using AU0.5 device due to over-concentration.	Reduce duration of centrifugation time during elution/concentration step.

Issue: High Non-specific binding	
Possible Cause	**Solution**
Antibody purified is not 100% the specific antibody of interest.	Use serum-free media for all hybridoma-based samples.
Insufficient washing.	Increase the volume of wash buffer used or number of wash steps. Supplement wash buffer with detergents such as 0.1% TWEEN®-20.

Performance

Purification with Amicon® Pro Affinity Concentration Kit – Protein G



Replicate purifications of IgG from rabbit serum using the Amicon® Pro Affinity Concentration Kit – Protein G. The numbered lanes are:
1 – lysate
2 – flowthrough fraction
3 – wash fraction
4 – eluted fraction
The prominent band visible in the wash fraction is albumin.

Ordering Information

Cat. No. BWEPG.

	No Devices	Amicon Pro + AU 0.5 mL with MWCO:				
		3k	10k	30k	50k	100k
Amicon® Pro Affinity Concentration Kit - Protein G	ACR5000PG	ACK5003PG	ACK5010PG	ACK5030PG	ACK5050PG	ACK5100PG
Amicon® Pro Affinity Concentration System 12 PK		ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Affinity Concentration System 24 PK		ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

The Amicon® Pro Affinity Concentration Kits contain reagents and devices sufficient for 12 standard reactions. Amicon® Pro devices are also sold separately in 12 and 24 packs.

Additional Reagents	Catalogue Number	Qty
Protein G Agarose	16-266	10 mL

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