One-Step Antibody Purification Kit

Introduction

BcMag™ One-Step Antibody Purification Kit is specifically designed for rapid small scale high-throughput purification of a variety of IgG species from serums such as humans, mice, rats, rabbits, goats, horses, guinea pig, pigs, hamsters, and donkeys. Unlike traditional bind-wash-elute affinity procedures, BcMag™ One-Step Antibody Purification Kit separates antibodies from serum by eliminating non-relevant proteins frequently present in large concentrations. The One-Step Antibody Purification beads bind non-antibody serum proteins such as albumin and transferrin, allowing the antibody to flow through in a moderate buffer ideal for storage and downstream uses. If the serum sample is not hemolyzed, it can be applied straight to the beads without the necessity for ammonium sulfate precipitation.

The One-Step Antibody Purification kit addresses the shortcomings of the commonly utilized immobilized Protein A and Protein G purification methods. IgG species and subclasses are selectively bound using Protein A and Protein G affinity techniques. The technique is often time-consuming and labor-intensive, requiring harsh elution conditions to disrupt the affinity connection. The purified antibody frequently requires dialysis or desalting before storage or use in downstream applications. The One-Step Antibody Purification technique does not require an elution step and instead employs a moderate purification buffer at physiological pH. Furthermore, because the purified antibody is in a buffer free of primary amines, it can be used directly in amine-reactive conjugation chemistries.

Magnetic resins have significant advantages over traditional chromatography, such as a column or non-magnetic resin. The magnetic bead-based format enables rapid high-yield processing of 96 samples in about 20 minutes, achieving more than 80% purities and recoveries of more than 90% for various IgG species. When using column-based technologies, processing multiple samples in academic research labs may necessitate a significant quantity of hand pipetting. This pipetting can discourage differences in the yield of target biomolecules between experiments and people. Staff and students may require extensive training and practice to produce constant protein yields. It is due to the numerous benefits of magnetic resins, such as their ease of use, rapid experimental protocols, suitability, and convenience for high throughput automated and miniaturized processing.

Feature and benefits:

- Efficient— recovery exceeds 90%, and purity exceeds 85%.
- Fast purification—One-Step and one-step high-throughput procedure
- Convenient—no columns, filters, or a laborious repeat of pipetting or centrifugation.
- Amine—free buffer does not require removal or neutralization.
- Robust—effective for IgG subclasses that bind poorly to Protein A or G
- Gentle— No harsh antibody elution conditions helps retain IgG activity.
- Regenerable—beads can be renewed and used for up to 3 purifications.

Workflow (Fig.1)

1. Add magnetic beads to the serum!
2. Mix the sample by pipetting up and down 25 times (one minute) or by vortex mixer.
3. Magnetic separation of the non-antibody serum protein-bound beads from the pure antibody.
4. Transfer the supernatant to a fresh tube.

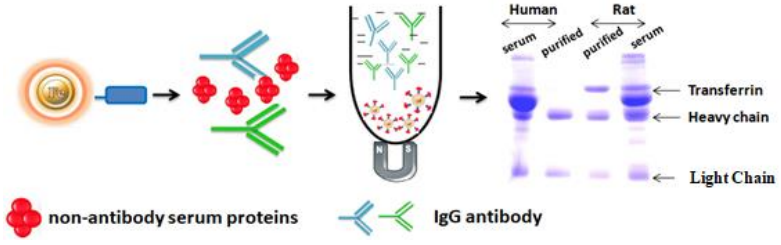

Fig.1 Workflow of one-minute antibody purification

Protocol

Note:

- The following protocol is an example. The beads and sample volume can be rational Scale-up (or down*).*
- Use 3ul of serum for every 40ul of beads. Impurities will result from inadequate bead use. Using too many beads may decrease the desired antibody's yield. IgG levels in serum are usually 10-15mg/ml; however, results may vary depending on species and sample preparation.
- Transferrin can be found in samples purified using beads from various species, including mouse and rat. Transferrin from these species has various properties and does not react with the beads like transferrin from other species does. Before purification, execute an ammonium sulfate precipitation to lower the presence of transferrin in the flow-through.

Equipment

Item	Source
Magnetic rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag 96-well Plate Magnetic Rack.	• BcMag 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ****** *IMPORTANT!* If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates must be ≥2.5mm.	
Addition items are required if using 96-well microplates	
Fisher Scientific™ Microplate Advanced Vortex Mixers	Fisher, Cat#:02-216-101
OHAUS Microplate Vortex Mixers	OHAUS, Cat#:30392160
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed≥ 800 rpm	
Clear Flat-bottom Non-Binding Assay Microplates	

Procedure

Important!

- The following protocol is optimized for the efficient clean-up of the 3µl serum sample. The procedure may need to be optimized if an alternative reaction scale is used.
- Shake or vortex the bottle to completely resuspend the magnetic beads before using.
- Do not allow the magnetic beads to sit for more than two minutes before dispensing.

1. Transfer 40µl beads to a new well of 96well PCR plate or 96-well microplates or 0.2ml PCR tube and add 3µl serum.
2. Mix the beads with the sample by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2000 rpm.

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3. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
4. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications.

Additional information:

Beads regeneration

1. If the bead has to be regenerated, add 50µL of 5M NaCl, and mix the beads with the sample by slowly pipetting up and down 25 times (one minute) or by vortex mixer 5 minutes at 2000 rpm. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear. Discard the supernatants.
2. Repeat step1 five times.
3. Resuspend the beads with 40µl of 25mM MES, PH6.5, and store at 4°C. The beads may be renewed three times without losing substantial selectivity.

Troubleshooting

Problem	Probable cause	Suggestion
The yield of the purified antibody is too low or undetectable	Sample devoid of antibody	• Ensure that the sample contains IgG by using another method, such as an ELISA or isotyping kit.
	The antibody of interest bound to the beads	• Ensure that the pH of the sample is between 6.5 and 7.0.
Observe multiple bands present on stained SDS-polyacrylamide gel	The sample contains salts greater than 25mM, and/or the pH is not neutral.	• Dialyze sample against a low-salt neutral buffer. • Make sure the pH of the sample is between 6.5 and 7.0.
A significant amount of antibodies was purified. However, no antibody of interest was found.	The concentration of antibodies of interest is low.	• Purify the antibody utilizing active affinity magnetic beads and a specific antigen.
Purified IgG is colored.	A sample of serum is hemolyzed.	Elimination of Hemolysis The following procedures can be used to decrease or eliminate hemolysis in serum samples. • Collect blood in the presence of an anticoagulant and centrifuged to remove red blood cells. • Collect blood with caution to avoid hemolysis. • Collect interstitial fluid instead of blood.

Related Products	
Glycoprotein and Antibody Conjugation Kit-I	Peptide conjugation buffer Kit-I
Glycoprotein and Antibody Conjugation Kit-II	Peptide conjugation buffer Kit-II
Protein A and G Purification Kit	Quick Albumin Removal Kit
Protein A Magnetic Beads Purification Kit	Quick HSA and IgG Depletion Kit
Protein G Magnetic Beads Purification Kit	Quick Antibody Purification Kit
Protein L Purification Kit	One-Step Antibody Purification Kit
Protein A and G Europium Fluorescent Magnetic Beads	Protein G Europium Fluorescent Magnetic Beads
Protein A and G Terbium Fluorescent Magnetic Beads	Protein G Terbium Fluorescent Magnetic Beads

Protein A and G Ruthenium Fluorescent Magnetic Beads	Protein G Ruthenium Fluorescent Magnetic Beads
Protein A Europium Fluorescent Magnetic Beads	Protein L Europium Fluorescent Magnetic Beads
Protein A Terbium Fluorescent Magnetic Beads	Protein L Terbium Fluorescent Magnetic Beads
Protein A Ruthenium Fluorescent Magnetic Beads	Protein L-Ruthenium Fluorescent Magnetic Beads

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