Application Note

Automated IgG Purification Using PureProteome™ Magnetic Beads With the KingFisher® Particle Processor

Introduction

As the demand for antigen-specific immunoglobulins increases in research, diagnostic and therapeutic settings, so increases the need for high reproducibility and batch-to-batch consistency of antibodies and antibody-based reagents. To meet requirements for both high throughput and low variability, many laboratories have turned to automated systems for antibody purification using protein A or protein G magnetic beads.

Compared to traditional purification methods using agarose either in batch mode or packed into spin columns, which require cumbersome centrifugation steps, automated magnetic bead processors are able to consistently process multiple samples at once while reducing hands-on time. Moreover, the process is highly reproducible and there is no risk of cross-contamination between samples.

Liquid handling and bead handling robots are the two types of systems commonly used for automating magnetic bead separation protocols. In liquid handling robots, magnetic separation is achieved by integrating an external 96-well microtiter magnetic rack to separate the beads from the liquid. Alternatively, bead handling robots such KingFisher® particle processors use magnetic rods covered with disposable plastic tips to move beads from one solution to the next.

PureProteome™ Protein A and G Magnetic beads provide a rapid and reproducible means to purify immunoglobulins from complex mixtures such as serum, plasma or cell culture supernatant samples. For IgG purification, the samples are mixed with PureProteome™ Protein G Magnetic Beads for a short period of time to bind the immunoglobulins. The beads are then isolated by magnetic separation, followed by several wash steps to remove unbound proteins. Finally, the bound proteins are eluted at high purity for use in further downstream applications. Here, we evaluated the effectiveness of PureProteome™ Protein G Magnetic Beads for IgG purification in a fully automated purification process using the KingFisher® Duo particle processor. To obtain the best results, parameters such as mixing speed, bead collection time and duration of binding and washing steps were optimized for use with PureProteome™ Protein G Magnetic Beads.

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Materials and Methods

Antibody Purification—Standard Manual Protocol

IgG purification from rabbit serum was performed using 100 µL of suspended PureProteome™ Protein G Magnetic Bead slurry (Cat. No. LSKMAGG10) in 1.5 mL microcentrifuge tubes (6 replicates). Phosphate-buffered saline (PBS) containing 0.01% Tween® 20 detergent (Cat. No. 655205) was used as binding/wash buffer. Using the PureProteome™ Magnetic Stand (Cat. No. LSKMAGS08), the beads were washed with 500 µL of binding buffer by vigorously vortexing for 10 seconds, collecting the beads on the magnet and removing the buffer with a pipette. 25 µL of rabbit serum diluted to a total volume of 200 µL with binding buffer was added to the beads, and the mixture was incubated at room temperature for 30 minutes with continuous, end–over-end mixing. The unbound fraction was discarded, and, as described above, the beads were washed three times with 500 µL of wash buffer using the PureProteome™ Magnetic Stand to capture beads. Finally, the elution of IgG was performed by adding 100 µL of 0.2 M glycine HCl, pH 2.5 to the beads and mixing at room temperature for 1–2 minutes. An additional elution was performed in a similar manner with 50 µL to achieve maximum yield. Both elutions were collected using the PureProteome™ Magnetic Stand into the same microcentrifuge tube and saved for further analysis.

Antibody Purification—KingFisher® Duo Protocol

Initially, all the reagents and samples were pipetted into the KingFisher® Duo plates (Microtiter Deepwell 96 plate and elution strips) and a protocol was set up on KingFisher® Duo System (Thermo Fisher) according to the plate layout and optimized conditions (mixing and collecting parameters for PureProteome™ magnetic beads) outlined in Table 1. Similarly to the manual protocol, 100 µL of suspended PureProteome™ Protein G Magnetic Bead slurry was used for IgG purification using KingFisher® Duo System. PBS containing 0.01% Tween® 20 detergent was used as binding/wash buffer and 25 µL of rabbit serum diluted in a total volume of 200 µL of binding buffer was pipetted into row D of the Microtiter Deepwell 96 plate and the binding step was performed for 30 minutes using medium mixing speed. Elution was carried out with 0.2 M glycine HCl, pH 2.5 in elution strips for better recovery of the sample. The protocol was executed and the plates were loaded onto the KingFisher® Duo System. After the run was completed, the plates were removed and the purified samples were collected for further analysis.

Table 1. Pipetting, mixing and collecting instructions for the purification protocol for KingFisher® Duo System

Plate/Row or Elution Strip	Row Name	Content	Volume	Mixing Time/Speed	Collecting Count/Time
1-A	Beads	Protein G Beads	100 µL	1 min/Medium	4/10 sec
1-B	Tip	12-Tip Comb	-	-	-
1-C	Equilibration	Binding Buffer	500 µL	1 min/Medium	4/10 sec
1-D	Bind	Sample + Binding Buffer	200 µL	30 min/Medium	4/10 sec
1-E	Wash 1	Wash Buffer	500 µL	1 min/Medium	4/10 sec
1-F	Wash 2	Wash Buffer	500 µL	1 min/Medium	4/10 sec
1-G	Wash 3	Wash Buffer	500 µL	1 min/Medium	4/10 sec
Elution Strip 1	Elution 1	Elution Buffer	100 µL	1 min/Medium	4/10 sec
Elution Strip 2	Elution 2	Elution Buffer	50 µL	1 min/Medium	4/10 sec

Protein Quantitation by Bradford Assay

10 µL of IgG standards and samples were added to a microtiter plate followed by 300 µL of Coomassie™ reagent. The reactions were mixed briefly on a plate shaker and analyzed by detecting the absorbance of the samples at 595 nm and the concentration was determined using the IgG standard curve.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Purified samples (10 µL of the eluted fractions) and control rabbit serum (5 µL of starting material) were reduced and denatured at 70 °C for 10 min and loaded onto 1 mm thick 4–12% gradient NuPAGE® Bis-Tris gels (Life Technologies). Proteins were separated at 200 V for 35 min. After electrophoresis, gels were removed from the cassette, briefly rinsed and washed 3 times with Milli-Q® water and stained with SimplyBlue™ SafeStain using the microwave protocol. Stained gels were then washed in Milli-Q® water for 10 minutes and imaged.

Results

Protein Yield

The purified samples obtained with the automated and manual protocols were analyzed by Bradford assay to determine protein yield. The results show that the total amount of IgG eluted using the KingFisher® system is similar to that of the manual process (Table 2).

	No. of Samples	µg IgG eluted	% CV
KingFisher® System	12	181.62	6.25
Manual	6	195.84	5.22

Table 2.
Bradford Assay Results: µg Total IgG eluted from PureProteome™ Protein G Magnetic Beads.

SDS-PAGE and Coomassie® Staining

The eluted fractions were also analyzed by SDS-PAGE for specificity of the elution and reproducibility. Visual inspection of the stained gel (Figure 1) demonstrate comparable purity and yield, indicating that PureProteome™ Protein G Magnetic Beads can be used to generate reproducible batches of IgGs using KingFisher particle processor.

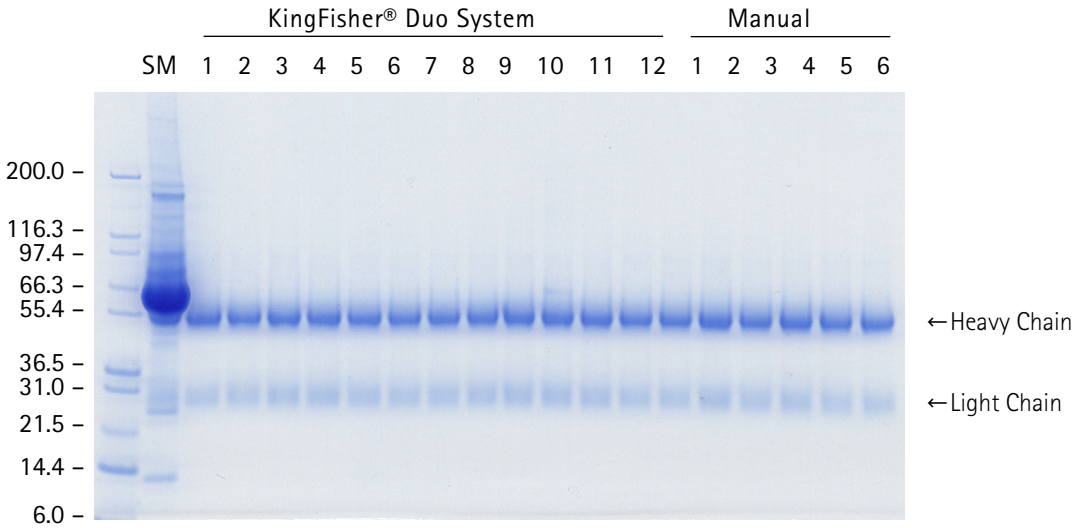


Figure 1. **SDS-PAGE gel: IgG purification from rabbit serum using PureProteome™ Protein G Magnetic Beads.** Elution of the antibody was performed using 0.2 M glycine HCl pH 2.5. Samples were separated by SDS-PAGE and bands were visualized by Coomassie® staining (Molecular weight standard is shown in leftmost lane; SM=starting material; remaining lanes are eluted fractions from KingFisher® Duo and eluted fractions from manual testing).

Discussion

We compared the overall binding efficiency and reproducibility of the PureProteome™ Protein G Magnetic Beads in an automated purification process using KingFisher® Duo particle processor to that of a standard protocol using PureProteome™ Magnetic Stand. The results from the Bradford Assay and SDS-PAGE gel confirm that PureProteome™ magnetic beads are very well suited for an automated purification process using King-Fisher® particle processor. Furthermore, our data indicate that the process is reproducible, demonstrates specific elution and is comparable to that of a standard protocol using PureProteome™ Magnetic Stand.

The paramagnetic properties of PureProteome™ magnetic beads make them ideal for automation. Because bead performance is critically dependent on using the right particle processing parameters, such as mixing speed, bead collection time and binding/washing duration, we have carefully optimized these parameters on the KingFisher® system specifically for PureProteome™ beads. Protein G was used in this study; however, the protocol can be implemented for PureProteome™ Protein A, Protein A/G Mix, Kappa and Lambda Magnetic Beads as well, depending on the IgG subtype or species being purified.

PureProteome™ magnetic beads are ideal for primary expression screening, small scale purification, and can be used on any manual or automated system for low, medium or high-throughput sample preparation, reducing hands-on time and costs.

Ordering Information

Description	Qty/Pk	Catalogue No.
PureProteome™ Protein A Magnetic Beads	2 x 1 mL 1 x 10 mL	LSKMAGA02 LSKMAGA10
PureProteome™ Protein G Magnetic Beads	2 x 1 mL 1 x 10 mL	LSKMAGG02 LSKMAGG10
PureProteome™ Protein A/G Mix Magnetic Beads	2 x 1 mL 1 x 10 mL	LSKMAGAG02 LSKMAGAG10
PureProteome™ Kappa Ig-Binder Magnetic Beads	2 x 1 mL	LSKMAGKP02
PureProteome™ Lambda Ig-Binder Magnetic Beads	2 x 1 mL	LSKMAGLM02
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08

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