Procedure

Sera-Mag™ SpeedBeads Magnetic Protein A/G Particles

Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles (Table 1) provide a fast and convenient method for both manual and automated magnetic isolation of proteins using affinity binding. The particles can be used for isolating antibodies from serum, cell culture supernatant or ascites, and for immunoprecipitation and co-immunoprecipitation of antigens from cell or tissue extracts. Bound antibodies or antigens are dissociated from the particles using an elution buffer.

The particles can be manually removed from the solution using a magnetic stand, or by automation using automated magnetic particle handling systems.

Table 1. Characteristics of Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles

Composition	Recombinant protein A/G covalently coupled to particle surface
Magnetization	Superparamagnetic (no magnetic memory)
Mean diameter	1 µm (nominal)
Concentration	10 mg/mL
Binding capacity	55–85 µg IgG bound per mg of particle
Particle density	~ 2.0 g/cm^3

Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles contain a recombinant Protein A/G (M_r ~50 500; apparent molecular weight by SDS-PAGE M_r ~40 000 to 45 000) that combines the IgG binding domains of both Protein A and Protein G.

Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a more general and convenient tool for investigating and purifying immunoglobulins. Also, Protein A/G binding to immunoglobulins is not as pH-dependant as Protein A.

Sera-Mag™ SpeedBeads Protein A/G particles are uniform, colloidally stable, monodispersed, non-porous superparamagnetic spheres made by a proprietary core-shell method.

The core is a carboxylate-modified particle made by free radical emulsion polymerization of styrene and acid monomer. Two layers of magnetite (Fe_3O_4) are coated onto this core particle, resulting in faster magnetic response times. The surface is chemically modified with a proprietary method to minimize nonspecific binding of proteins. Finally, Protein A/G is covalently bound to the particle surface.

The particles are supplied at 1% solids (10 mg/mL) in 0.05 % sodium azide and are available in 1 mL, 5 mL and 100 mL package sizes.

Important information before using Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles

- Do not centrifuge, dry or freeze the magnetic particles. Centrifuging, drying or freezing will cause the particles to aggregate and lose binding activity.
- We recommend thoroughly mixing (vortex, roll or sonicate) magnetic protein A/G particles before use. Sonication is the preferred method to resuspend the particles thoroughly and efficiently.
- Sonication with a probe type ultrasonicator is recommended to resuspend particles after long term storage, and washing steps.
- To minimize protein degradation, include protease inhibitors in preparation of cell lysates.
- A low-pH elution may be used for single-use applications. Optimal time for low-pH elution is 10 minutes. Exceeding 10 minutes may result in nonspecific binding and yield reduction.
- When using rabbit antibodies (primary or secondary) in downstream Western blot applications, perform elution in SDS-PAGE sample buffer at room temperature. For all other antibody species, boiling the particles in SDS-PAGE sample buffer is acceptable for single-use applications. Boiling will cause particle aggregation and loss of binding activity.
- Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles are compatible with small-scale antibody purification and immunoprecipitation and analyses by Western blot and mass spectrometry.

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Procedure for manual antibody purification

Additional materials required

- 1.5 mL microcentrifuge tubes.
- Sample: serum, concentrated cell culture supernatant or concentrated ascites.
- Binding/Wash buffer: Tris-buffered saline (25 mM Tris, 0.15 M NaCl, pH 7.5) containing 0.05 % Tween™-20 detergent.
- Elution buffer: 0.1 M glycine, pH 2–3.
- Neutralization buffer: High-ionic strength alkaline buffer such as a 1 M phosphate or 1 M Tris; pH 7.5–9.
- Magnetic stand (e.g., MagRack 6, Cytiva code number 28948964).

Antibody purification from serum, cell culture supernatant, or ascites

Note: To ensure homogeneity, mix the particles thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

1. Place 50 µL (0.50 mg) of Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles into a 1.5 mL microcentrifuge tube. Add 150 µL of binding/wash buffer to the particles and gently vortex to mix.
2. Place the tube into a magnetic stand to collect the particles against the side of the tube. Remove and discard the supernatant.
3. Add 1 mL of binding/wash buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect particles with magnetic stand, then remove and discard the supernatant.
4. Dilute 10 µL of sample with 490 µL binding/wash buffer.

Note: Sample volume can be modified according to user preference. If the sample volume is < 500 µL dilute it to a final volume of 500 µL with binding/wash buffer.

5. Add the diluted sample to the tube containing prewashed magnetic particles and gently vortex or invert to mix.
6. Incubate the samples at room temperature with mixing for 1 hour.
7. Collect the particles with a magnetic stand, then remove and discard the supernatant.
8. Add 500 µL of binding/wash buffer to the tube, mix well, collect the particles with a magnetic stand and discard the supernatant. Repeat this wash twice.
9. Add 100 µL of elution buffer to the tube, mix well and incubate 10 minutes at room temperature with occasional mixing.
10. Collect the particles with a magnetic stand and then remove and save the supernatant that contains the eluted antibody. To neutralize the low pH, add 10 µL of neutralization buffer for each 100 µL of eluate.

Note: 50 µL is the minimum volume of particles recommended for antibody purification.

Procedure for automated antibody purification

Additional materials required

1. KingFisher™ Flex with 96 Deep Well head (Thermo Scientific product number 5400630) or KingFisher™ 96 (Thermo Scientific product number 5400500).
2. KingFisher™ Flex Microtiter Deepwell 96 plate, V-bottom (Thermo Scientific product number 95040450).
3. KingFisher™ Flex 96 tip comb for Deep Well Magnets (Thermo Scientific product number 97002534).
4. Binding/wash buffer: Tris-buffered saline containing 0.05% Tween™-20 detergent).
5. Elution buffer: 0.1 M glycine, pH 2–3.
6. Neutralization buffer: High-ionic strength alkaline buffer such as a 1 M phosphate or 1 M Tris; pH 7.5–9.

Preparation of instrument and plate set-up

Note: The following protocol is designed for general use with the KingFisher™ Flex or KingFisher™ 96 Instrument. The protocol can be modified according to customer needs using the Thermo Scientific BINDIT™ software provided with the instrument.

1. Download the "Antibody Purification" protocol from the Thermo Fisher Scientific website (https://www.thermofisher.com/home/life-science/dna-rna-purification-analysis/automated-purification-extraction/kingfisher-systems.html) into the BINDIT™ software on an external computer.
2. Transfer the protocol to the KingFisher™ Flex or KingFisher™ 96 from an external computer. See BINDIT™ Software User Manual for detailed instructions on importing protocols.
3. Set up the plates according to Table 2.

Table 2. Pipetting Instructions for the Antibody Protocol Using the Microtiter Deep Well 96 Plates

Plate	Plate name	Content	Volume
1	Particles	Protein A/G particles	50 µL
		Binding/Wash buffer	150 µL
2	Particle wash	Binding/Wash buffer	1000 µL
3	Bind	Sample	10 µL
		Binding/Wash buffer	490 µL
4	Wash 1	Binding/Wash buffer	500 µL
5	Wash 2	Binding/Wash buffer	500 µL
6	Wash 3	Binding/Wash buffer	500 µL
7	Elution	Elution buffer	100 µL
8	Tip plate	KingFisher™ Flex 96 tip comb for Deep Well magnets	–

Notes

- If using less than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the particles to plate 1.
- Combine the Tip Comb with a Deep Well 96 plate. See KingFisher™ Flex or KingFisher™ 96 user manual for detailed instructions.
- Sample volume can be modified according to user preference. If the sample volume is < 500 µL dilute it to a final volume of 500 µL with binding/wash buffer.

Executing the antibody purification protocol on the KingFisher™ Flex

1. Select the protocol using the arrows on the instrument key pad and press Start. See KingFisher™ Flex User Manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load the plates into the KingFisher™ Flex according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing Start.
4. After the samples are processed, remove the plates as instructed by the instrument's display. Press Start after removing each plate.
5. Press Stop after all plates are removed.
6. Upon completion, if desired, neutralize the low pH by adding 10 µL of neutralization buffer for each 100 µL of eluate.

Procedure for manual immunoprecipitation (IP)

Additional materials required

- 1.5 mL microcentrifuge tubes.
- Binding buffer: Tris-buffered saline (25 mM Tris, 0.15 M NaCl, pH 7.5) containing 0.05% Tween™-20 detergent.
- Wash buffer: 25 mM Tris, 0.65 M NaCl, 0.05% Tween™-20 detergent, pH 7.5.
- Low-pH elution buffer: 0.1 M glycine, pH 2–3.
- Alternative elution buffer: SDS-PAGE reducing sample buffer.
- Antibody for immunoprecipitation.
- Antigen sample.
- Cell lysis buffer (used to adjust IP reaction volume).
- Neutralization buffer: High-ionic strength alkaline buffer such as a 1 M phosphate or 1 M Tris; pH 7.5–9.

Immunoprecipitation

Note: This protocol is a general guideline for immunoprecipitation and will require optimization for each application.

1. Combine the antigen sample with 10 µg of antibody. Adjust the reaction volume to 500 µL with the cell lysis buffer. Incubate the reaction for 1 to 2 hours at room temperature or overnight at 4°C with mixing.
2. Place 25 µL (0.25 mg) of Sera-Mag™ SpeedBeads Protein A/G Magnetic Particles into a 1.5 mL microcentrifuge tube.
3. Add 175 µL of wash buffer to the particles and gently vortex to mix.
4. Place the tube into a magnetic stand to collect the particles against the side of the tube. Remove and discard the supernatant.
5. Add 1 mL of wash buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect particles with magnetic stand. Remove and discard the supernatant.
6. Add the antigen sample/antibody mixture to a 1.5 mL microcentrifuge tube containing prewashed particles and incubate at room temperature for 1 hour with mixing.
7. Collect the particles with a magnetic stand and then remove the flow-through and save for analysis.
8. Add 500 µL of wash buffer to the tube and gently mix. Collect the particles and then discard the supernatant. Repeat this wash twice.
9. Add 500 µL of purified water to the tube and gently mix. Collect the particles on a magnetic stand and discard the supernatant.
10. Low-pH Elution: Add 100 µL of low-pH elution buffer to the tube. Incubate the tube at room temperature with mixing for 10 minutes. Magnetically separate the particles and save the supernatant containing target antigen. To neutralize the low pH, add 10 µL of neutralization buffer for each 100 µL of eluate.

Alternative elution: Add 100 µL of SDS-PAGE reducing sample buffer to the tube and heat the samples at 96°C to 100°C in a heating block for 10 minutes. Magnetically separate the particles and save the supernatant containing target antigen.

Note: If you will be performing a Western blot using rabbit antibodies (primary or secondary) do not heat the samples. Incubate at room temperature for 10 minutes with mixing.

Procedure for automated immunoprecipitation

Additional materials required

- KingFisher™ Flex with 96 Deep Well head (Thermo Scientific product number 5400630) or KingFisher™ 96 (Thermo Scientific product number 5400500).
- KingFisher™ Flex Microtiter Deepwell 96 plate, V-bottom (Thermo Scientific product number 95040450).
- KingFisher™ Flex 96 tip comb for Deep Well magnets (Thermo Scientific product number 97002534).
- 1.5 mL microcentrifuge tubes.
- Binding buffer: Tris-buffered saline containing 0.05 % Tween™-20 detergent.
- Wash buffer: Tris-buffered saline containing 0.05 % Tween™-20 detergent and 0.5 M NaCl.
- Low-pH elution buffer: 0.1 M glycine, pH 2–3.
- Alternative elution buffer: SDS-PAGE reducing sample buffer.
- Antigen sample.
- Cell lysis buffer (used to prepare the antigen sample).
- Neutralization buffer: High-ionic strength alkaline buffer such as a 1 M phosphate or 1 M Tris; pH 7.5–9).
- Magnetic stand (e.g., MagRack 6, Cytiva code number 28948964).

Instrument preparation and plate set-up

Note: The following protocol is designed for general use with the KingFisher™ Flex or KingFisher™ 96 Instrument. The protocol can be modified according to your needs using the BINDIT™ Software provided with the instrument.

1. Combine antigen sample with 2–10 µg of immunoprecipitation antibody per sample. Incubate 1–2 hours at room temperature or overnight at 4°C with mixing.
2. Enter the "Immunoprecipitation" protocol from Table 3 into the BINDIT™ Software on an external computer.
3. Transfer the protocol to the KingFisher™ Flex or KingFisher™ 96 from an external computer. See BINDIT™ Software User Manual for detailed instructions on importing protocols.
4. Set up plates according to Table 3.

Table 3. Pipetting instructions for the immunoprecipitation protocol using the Microtiter Deep Well 96 Plates

Plate	Plate name	Content	Volume	Time/speed
1	Particles	Protein A/G particles Binding buffer	50 µL 150 µL	5 s
2	Particle wash	Binding buffer	1000 µL	1 minute/ slow
3	Bind	Antibody/ Antigen sample	500 µL	1 minute/ slow
4	Wash 1	Binding/Wash buffer	500 µL	30 s/slow
5	Wash 2	Binding/Wash buffer	500 µL	30 s/slow
6	Wash 3	Ultrapure water	500 µL	30 s/slow
7	Elution	Elution buffer	100 µL	10 minute/ slow
8	Tip plate	KingFisher™ Flex 96 tip comb for Deep Well magnets	–	10 minute/ fast

Notes

- If using less than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure particle homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the particles to plate 1.
- Combine the Tip Comb with a Deep Well 96 plate. See KingFisher™ Flex or KingFisher™ 96 user manual for detailed instructions.
- The particles can be eluted into 100 µL of 0.1 M glycine, pH 2–3 or 100 µL SDS-PAGE reducing sample buffer. If using SDS-PAGE reducing sample buffer in a heated elution, install the KingFisher™ Flex or 96 Heating Block (see manual for proper installation) to heat samples at 96°C to 100°C for 10 minutes.
- If you select SDS-PAGE reducing sample buffer for elution and will be performing a Western blot using rabbit antibodies (primary or secondary), do not heat the samples. Incubate at room temperature for 10 minutes.
- If low-pH elution buffer is selected for elution, neutralize the pH using 10 µL neutralization buffer for each 100 µL of eluate upon run completion.
- To limit evaporation, select "Mix" and "Slow" speed under the subheading "Heating Action".

Executing automated immunoprecipitation protocol

1. Select the protocol using the arrows on the instrument key pad and press Start. See the KingFisher™ Flex User or KingFisher™ 96 User Manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing Start.
4. After the samples are processed, remove the plates as instructed by the instrument's display. Press Start after removing each plate. Press Stop after all the plates are removed.

Troubleshooting

Problem	Possible cause	Solution
Low amount of protein was recovered	The protein degraded	Add protease inhibitors
	Insufficient magnetic particles used	Increase the amount of magnetic particles used for capture
	Insufficient target protein present in sample	Increase amount of antigen sample
Protein does not elute	Elution conditions are too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Bands at M_r 50 000 appear on Western blot	Elution conditions are too stringent	Perform elution at room temperature
Multiple, nonspecific bands appear in eluted sample	Nonspecific protein binding to the magnetic particles	Add 50–200 mM NaCl to the binding/wash and/or elution buffers
Recovered protein was inactive	Elution conditions are too stringent	Use a milder elution buffer
Magnetic particles aggregate	Magnetic particles were frozen or centrifuged	Handle the particles as directed in the instructions
	Buffer is incompatible with magnetic particles	

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