MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

Protein L Magnetic Beads

1 Contents

Cat. No.	Product Name	Package
HY-K0205-1 mL	Protein L Magnetic Beads	1 mL
HY-K0205-5 mL	Protein L Magnetic Beads	1 mL × 5

2 General Information

MCE Protein L Magnetic Beads provide a fast and convenient method for magnetic isolation of proteins using affinity binding. MCE Protein L Magnetic Beads are typically used for isolating antibodies from serum, cell culture supernatant or ascites and for Immunoprecipitation and Co-Immunoprecipitation of antigens from cell or tissue extracts.

The MCE Protein L Magnetic Beads contain a recombinant Protein L that binds to antibody species and subtypes with appropriate Kappa light chains, while Lambda light chains and some Kappa light chains do not bind, making it a more general and convenient tool for investigating and purifying immunoglobulins.

3 Characteristics

Composition	Recombinant Protein L monolayer covalently coupled to a blocked magnetic beads surface
Magnetization	Superparamagnetic
Mean Diameter	2 μm
Bead Concentration	10 mg/mL
Binding Capacity	≥1 mg/mL

4 General Protocol

Recommended Buffer

Binding/Wash Buffer	PBST: 1× PBS + 0.5% Triton X-100, pH 7.4
Elution Buffer	0.15 M Glycine, 0.5% Triton X-100 or Tween-20, pH 2.5-3.1

1. Preparation of Magnetic Beads

1.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

1.2 Transfer 25-50 μL of Protein L Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

1.3 Add 400 μL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Hereinafter referred to as magnetic separation). Remove and discard the supernatant. Repeat this step for 2 times.

2. Binding of Antibody

2.1 Dilute antibody to the final concentration of 5-50 μg/mL with binding/wash buffer. The optimal amount of Ab may be adjusted as required.

2.2 Add 400 μL of diluted Ab to the Protein L Magnetic Beads. Rotate tube for 30 minutes at room temperature or 2 hours at 4°C.

2.3 Perform magnetic separation. Transfer the supernatant into a new tube for futher analysis, if desired. The supernatant is the non-binding fraction.

2.4 Add 400 μL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 4 times.

Note: During the binding process, it won't affect the result if magnetic beads occasionally aggregated together.

3. Immunoprecipitation of Target Antigen

3.1 Remove the tubes from the magnetic separator and add your sample containing the antigen (Ag) (typically 5-50 μg in 400 μL binding/wash buffer) and gently pipette to resuspend the Protein L Magnetic Beads-Ab complex.

3.2 Incubate with rotation for 30 minutes at room temperature or 2 hours at 4°C to allow Ag to bind to the Protein L Magnetic Beads-Ab complex.

Note: Depending on the affinity of antibody, it may be necessary to increase the incubation time for optimal binding.

3.3 Perform magnetic separation. Remove and discard the supernatant.

3.4 Wash the Magbeads-Ab-Ag complex 5 times using 400 μL binding/wash buffer for each wash. Perform magnetic separation between each wash, remove supernatant and resuspend by gentle pipetting.

3.5 Resuspend the Protein L Magnetic Beads-Ab-Ag complex in 400 μL binding/wash buffer and transfer the bead suspension into a clean tube. This is recommended to avoid co-elution of the proteins bound to the tube wall.

4. Elution

This is a non-denaturation elution method.

4.1 Perform magnetic separation and remove the supernatant. Add 400 μL of binding/wash buffer into the tube and rotate for 5 minutes. Perform magnetic separation for 1 minute and remove the supernatant. Then add 25-50 μL elution buffer into the tube with magnetic beads-Ab-Ag complex, rotate for 5 minutes.

4.2 Perform magnetic separation, collect the supernatant.

4.3 The final solution can be used as samples for denaturing SDS-PAGE, or the elution can be adjusted to neutral pH with neutralization buffer immediately and can be used for further analysis.

5 Storage

Stored at 4°C, and is stable for up to 2 years.

6 Precautions

1. The pH of Protein L Magnetic Beads is 6-8.

2. Do not centrifuge, dry or freeze the magnetic beads.

3. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

7 Troubleshooting

Problem	Possible Cause	Solution
Low binding efficiency of antibody and magnetic beads	The binding efficiency of magnetic beads and antibody depends on the origin and subtype of the antibody	Check the affinity of antibody and the protein A/G matrix in the appendix
	The antibody subtype and protein L show low affinity	Elongate the incubation time of antibody and magnetic beads
		Improve the pH vaule of binding buffer (8-9) and reduce the ion strength (25-100 mM NaCl).
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions.
	Buffer was incompatible with magnetic beads	
Multiple nonspecific bands	Nonspecific proteins bound to the magnetic beads	Add 50-350 mM of NaCl to the binding/washing and elution buffers.
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer or use more stringent elution buffer.
Low amount of protein was recovered	The protein degraded	Add protease inhibitors.
	Not enough magnetic beads were used	Increase the amount of magnetic beads used for capture.
	Sample had an insufficient amount of target protein	Increase amount of antigen sample.

Appendix: Binding Affinity of Protein A & Protein G & Protein A/G & Protein L for Different Antibodies and Isotypes

Species		Protein A	Protein G	Protein A/G	Protein L
Human	IgG	+++	+++	+++	+++*
	IgG_1	++++	++++	++++	+++*
	IgG_2	++++	++++	++++	+++*
	IgG_3	-	+++	+++	+++*
	IgG_4	++++	++++	++++	+++*
	IgA	+	-	+	+++*
	IgA_1	+	-	+	+++*
	IgA_2	+	-	+	+++*
	IgD	+	-	+	+++*
	IgE	++	-	++	+++*
	IgM	+	-	+	+++*
	Fab	+	+	+	+++*
	ScFv	+	-	+	+++*
Mouse	IgG	+++	+++	++++	+++*
	IgG_1	+	++++	+++	+++*
	IgG_{2a}	+++	+++	+++	+++*
	IgG_{2b}	+++	+++	+++	+++*
	IgG_3	++	+++	+++	+++*
	IgM	-	-	-	+++*
Rat	IgG	+	++	++	+++*
	IgG_1	+	+	++	+++*
	IgG_{2a}	+	++++	+++	+++*
	IgG_{2b}	+	++	+	+++*
	IgG_{2c}	++	++	+++	+++*
	IgG_3	+	++	++	+++*
Cow	IgG	+	+++	+++	-
	IgG_1	+	+++	+++	-
	IgG_2	++	+++	+++	-
Goat	IgG	++	+++	++++	-
	IgG_1	+	+++	+++	-
	IgG_2	+++	+++	+++	-
Sheep	IgG	+	++	+++	-
	IgG_1	+	++	+++	-
	IgG_2	+++	+++	+++	-
Horse	IgG	++	++++	++++	?
Rabbit	IgG	+++	+++	+++	+*
Guinea Pig	IgG	+++	+	+++	?
	IgG_1	++	+	++	?
	IgG_2	++	+	++	?
Hamster	IgG	+	++	++	+*
Pig	IgG	+++	++	+++	+++*
Donkey	IgG	++	+++	+++	?
Cat	IgG	+++	+	++	?
Dog	IgG	++	+	+++	?
Monkey	IgG	++++	++++	++++	?
Chicken	IgG	-	-	-	-
Koala	IgG	-	+	+	?
Llama	IgG	-	+	+	?

Notes: "+"= weak binding, "+++"= medium binding, "++++" = strong binding, "-" = no binding.
"*": the binding strengths for Protein L refer only to antibody species and subtypes with appropriate kappa light chains. Lambda light chains and some kappa light chains do not bind.