

## **User Manual**

# BeaverBeads<sup>TM</sup> Protein A (or A/G) Immunoprecipitation

### **Product Introduction**

BeaverBeadsTM Protein A / G Matrix beads immunoprecipitation product enables Protein A / G to cover superparamagnetic microspheres with high density by using Beaver biological nano- technology. Comparing to similar products currently on the international immunomagnetic beads market, it has more antibody binding points, less usage of beads and low binding rate with other protein, so it makes immunoprecipitation experiments more efficient. Each milliliter immunoprecipitation beads can combine with human IgG for more than 300µg, and a single precipitation reaction only need 25µL beads. The super-large specific surface area provided by the micrometer beads can significantly reduce the equilibrium time required for adsorption of antibody and antigen. The antibody adsorption process can be completed within 15 min and the antigen precipitation process is completed within 30 min. Short operating time can avoid target protein hydrolysis. ensuring the activity of the target protein and integrity of protein complex.

This product can be widely used in cell lysate, cell secretion supernatant, blood plasma, ascites, tissue culture supernatant and other samples of antigen immunoprecipitation reaction. The operator can refer to the operating instructions, the data in Table 1 can be used to understand the binding capacity between different species and subtypes of antibodies and Protein A beads, Protein A/G.

### Product Information

TM BeaverBeads Protein A (or A/G) Immunoprecipitation			
Combing Human IgG	Protein A	0.4~0.5 mg/mL	
capability	Protein A/G	0.5~0.6 mg/mL	
Preservation temperature	2~8°C		
storage life	1 year		

#### **Notes**

1. Be sure to read this operating instruction carefully before performing an immunoprecipitation operation.

2. This product must be used with the magnetic separator.

- 3. Beads should be fully oscillated before use.
- 4. The beads should be stored in the storage solution to prevent drving.
- 5. Do not freeze or centrifuge the beads to avoid irreversible aggregation.

6. To ensure the best results, select a specific antibody for immunoprecipitation.

7. The operator can detect the binding situations of antibodies, antigens and beads using the antibody binding reaction procedure and the supernatant collected in the antigen binding reaction step according to the actual requirements.

Service@beaverbio.com 8. For IP experiments, the affinity of different types of antibodies to antigen binding is different, and the binding of the antibody to the antigen will also be affected by the binding buffer and the wash buffer.

9. The coated protein A / G on the surface of this bead has few protein shedding situations under extreme conditions (such as low pH, heat treatment), but it is not advisable for the operator to use a protein with a molecular weight of about 130 kD Immunoprecipitation experiments.

10. This product is for research use only.

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#### Q1 : How to improve the binding efficiency of antibody and magnetic beads?

A1: The binding efficiency of beads and antibody is related to the source and subtype of the antibody. Please check the type of the antibody and affinity of the antibody to the Protein A / G ligand (Table 1). If the affinity is low, operator can increase the incubation time of antibody and magnetic beads (30 ~ 120 min), raise the binding buffer to pH 8~ pH 9 and reduce the ionic strength (25 ~ 100 mM NaCl) and other methods to improve the affinity efficiency.

#### Q2: How to improve the specificity of magnetic beads in immunoprecipitation?

A2: Antibodies can be incubated with the sample to form an antibody-antigen complex first, and use the protein A/G beads to capture the complex later. This method can improve the efficiency of antibody and antigen binding, and reduce the mixing time between the beads and the sample, thereby enhancing the specificity of the precipitated product. This method is also recommended for protein/nucleic acid coprecipitation or chromatin immunoprecipitation.

#### Q3: How to avoid the aggregation of the beads in the storage or process of the use?

A3: Magnetic beads should be stored at 2 ~ 8 °C, when using, the pollution and the dry circumstance should be avoided to cause the irreversible aggregation. Magnetic beads occurring aggregation is a normal phenomenon in the low pH elution buffer, but it does not affect the normal use. The addition of non-ionic detergents (such as NP-40, Tween-20 or Triton X-100) with a final concentration of 0.1% (v / v) in the Binding buffer and Elution buffer can effectively prevent the accumulation of beads. The beads subjected to low pH elution can be washed to neutral with binding buffer and then suspended with a Tris buffer (pH 7.5) containing 0.1% (y / y) Tween-20 and treated with ultrasonic bath 2 min. the magnetic beads can be restored to a uniform state, the above treatment does not affect the antibody binding efficiency of magnetic beads.

#### Q4: How to solve the problem of the beads easily adhere to the tube wall?

A4: It is recommended to use low-adsorption consumables for magnetic bead operation. In addition, a nonionic detergent was added to the buffer solution of 0.01% to 0.1% (v / v) (Such as NP-40, Tween-20 or Triton X-100) can effectively reduce the adhesion of the beads to the consumables.

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#### Q5: How to solve the problem of agglomeration of magnetic beads during the usage?

A5: When using beads, if agglomeration occurs, it's generally difficult to break down by just hand vibration, easily lead to uneven distribution. The cause for this problem is that the beads are placed in the magnetic field for too long so that the beads are firmly bonded together. Put within ultrasonic bath for 2 min can efficiently disperse and redistribute the beads, but please note that ultrasonic treatment will also make the antibody off which is captured by beads in the sample solution , so it's not recommended to use this method after adding sample or before the elution process.

# Table 1: Comparison of Antibody Affinity between Protein A and Protein A / G with Different Sources and Types

Pecie	Antibody Classs	Protein A/G	Protein A
	Total IgG	+++++	+++++
	IgG1, IgG2	++++	+++++
	IgG <sub>3</sub>	+++++	+
	IgG <sub>4</sub>	+++++	+++++
	IgM	-	-
Human	IgD	-	-
	IgA	+	+
	IgA <sub>1</sub> , IgA <sub>2</sub>	+	+
	IgE	+++	+++
	Fab	-	-
	ScFv	-	-
	Total IgG	+++++	+++++
	IgM	-	-
Mouse	IgG <sub>1</sub>	+++	+
Mouse	IgG <sub>2a</sub>	+++	+++
	IgG <sub>2b</sub>	+++	+
	IgG <sub>3</sub>	+++	+++++
	Total IgG	+++	+
	IgG1	+++	+
Rat	IgG <sub>2a</sub>	++++	-
	IgG <sub>2b</sub>	+	-
	IgG <sub>2c</sub>	++++	+++
	Total IgG	++++	+
Cow	IgG <sub>1</sub>	++++	+
	IgG <sub>2</sub>	++++	+++++
	Total IgG	++++	+
Goat	IgG <sub>1</sub>	++++	+
	IgG <sub>2</sub>	++++	+++++
	Total IgG	++++	+
Sheep	IgG <sub>1</sub>	++++	+
	IgG <sub>2</sub>	++++	+++++
	Total IgG	++++	+
Horse	IgG(ab), IgG(c)	+	+
	IgG(T)	+++++	-
Rabbit	Total IgG	+++++	+++++
Guinea	Total IgG	+++++	+++++
Hamster	Total IgG	+++	+++

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Pig	Total IgG	+++++	+++++
Donkey	Total IgG	+++++	+++
Cat	Total IgG	+++++	+++++
Dog	Total IgG	+++++	+++++
Monkey	Total IgG	+++++	+++++
Chicken	Total IgY	-	-

Note: "+"=weak binding, "+++"=medium binding, "+++++"=strong binding, "-"=no binding

#### Immunoprecipitation beads and related products information

Cat No.	Prosuct Name	Specification	Bead Diameter	Human IgG binding
22202-20	BeaverBeads <sup>TM</sup> Protein A/G	20rnxs	2 µm	0.5~0.6 mg/mL
22202-100	BeaverBeads <sup>TM</sup> Protein A/G	100 rnxs	2 µm	0.5~0.0 mg/m∟
22203-20	BeaverBeads <sup>TM</sup> Protein A	20 rnxs	2 µm	0.4~0.5 mg/mL
22203-100	BeaverBeads <sup>TM</sup> Protein A	100 rnxs	2 µ11	0.4~0.5 mg/m∟

Cat No.	Magnetic Separator		Note
60201	Magnetic Separator Stand 2/15	2/15 mL	Suitable for 1.5 mL, 2 mL EP tube and 15 mL centrifuge tube
60203	Magnetic Separator Stand 50	50 mL	Suitable for regular 50 mL centrifuge tubes
60302	Magnetic Separator Stand 96-I	96-1	Suitable for conventional 96-well plate, PCR plate, 8-well or 12-well PCR tube etc.
60303	Magnetic Separator Stand 96-II	96-11	Suitable for 96-well PCR plates (20 to 200 µL experimental system)

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