MagListo™ Protein A Kit

[Cat. No.] K-7720

Introduction

MagListo™ Protein A Kit allows rapid and easy purification of antibodies through magnetic separation by *AccuNanoBead*™ Protein A Magnetic NanoBeads and buffers. *AccuNanoBead*™ Protein A Magnetic NanoBeads are silica beads conjugated with high purity (>95%) Protein A, which allow specific recognition of antibodies and purification of 0.8 mg of human IgG per 1 ml of bead solution. *MagListo*™ kit can also be used together with *ExiProgen*™ Consumable SET (Cat. No. KA-3001) by connecting to the *ExiProgen*™ instrument for automated protein purification. This *MagListo*™ kit can be used for antibody purification, immunoprecipitation, antigen-antibody interaction, and cell separation.

Features & Benefits

- Outstanding efficiency: Minimized loss through a strong magnetism out of magnetic nanobeads.
- Large binding capacity: Magnetic nanobeads with an average diameter of 400 nm gives rise to large surface areas for binding.
- High specificity: Reduced non-specific binding by using homogeneous spherical magnetic nanobeads.

Components

Components	Amount
AccuNanoBead™ Protein A Magnetic NanoBeads	40 mg/ml x 1 ea
Binding & Washing buffer	20 ml x 2 ea
Elution buffer	1 ml x 2 ea
Neutralization buffer	1 ml x 1 ea

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

AccuNanoBead™ Protein A Magnetic NanoBeads	
Composition	Silica based magnetic nanobeads
Binding capacity	> 0.8 mg of human IgG/ml of beads
Size	Average 400 nm
Concentration	40 mg/ml

Storage Buffer

AccuNanoBead™ Protein A Magnetic NanoBeads are supplied as a 4% (v/v) suspension in 1X storage buffer (phosphate buffered saline, pH 7.4, 0.02% Tween-20, and 0.1% NaN_3).

Storage

Store at 2-8°C.

Precautions

- Do not freeze and vigorously vortex *AccuNanoBead*™ Protein A Magnetic NanoBeads.
- An exact protocol may need to be optimized by the user.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

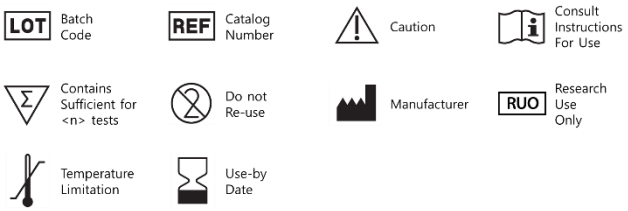
Ordering Information

Description	Cat. No.
MagListo™ Protein A Kit	K-7720

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



Experimental Procedures

Steps		Procedure Details
Antibody purification		
1	 **Equilibrating magnetic nanobeads**	1. Resuspend *AccuNanoBead*™ Protein A Magnetic NanoBeads by gently vortexing. 2. Transfer 100 µl of magnetic nanobeads to a 1.5 ml tube and place the tube on a Neodymium (Nd) magnet for 1 min. 3. Remove the supernatant. 4. Equilibrate by adding 1 ml of Binding & Washing buffer to the bead slurry and mix briefly. 5. Place the tube on the Nd magnet for 1 min and remove the supernatant. 6. Repeat step 4 and 5 once more.
2	 **Sample Binding**	7. Load about 500 µl of the sample containing antibody and 500 µl of Binding & Washing buffer onto the pre-equilibrated magnetic nanobeads. 8. Incubate in a rotator for 1 hr at room temperature. *** Note:** Make sure that the magnetic nanobeads are evenly resuspended. This is important for an efficient purification. 9. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** Supernatant from this step is the **Unbound sample** for checking the binding efficiency.
3	 **Washing magnetic nanobeads**	10. Add 500 µl of Binding & Washing buffer and wash the magnetic nanobeads by gently pipetting. 11. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** Supernatant from this step is the **Washing sample** for checking the washing conditions. 12. Repeat step 10 and 11 once more. *** Note:** After the final wash, the remaining Binding & Washing buffer should be removed completely.
4	 **Elution**	13. Add 100 µl of Elution buffer to elute antibody from magnetic nanobeads and gently mix. 14. Incubate for 1 min at room temperature. 15. Place the tube on the Nd magnet for 1 min and transfer the elution fraction to a new tube. *** Note:** For a better yield, repeat the elution step once more or increase elution buffer volume. 16. Add 10 µl (10% of eluate‡) of Neutralization buffer† to the elution fraction. *** Note:** Elution fraction from this step is the **Elution sample** for checking the target proteins. † User may utilize other buffers for neutralization depending on the purpose of the experiment. ‡ Elution buffer and Neutralization buffer should be mixed at a ratio of 9:1. The pH of mixture should be between 7-8. This only applies when using Elution buffer and Neutralization buffer provided in the kit.

Immunoprecipitation		
1	 **Equilibrating magnetic nanobeads**	1. Resuspend *AccuNanoBead*™ Protein A Magnetic NanoBeads by gently vortexing. 2. Transfer 50 µl of magnetic nanobeads to a 1.5 ml tube and place the tube on a Neodymium (Nd) magnet for 1 min. 3. Remove the supernatant. 4. Equilibrate by adding 250 µl of Binding & Washing buffer to the bead slurry and mix briefly. 5. Place the tube on the Nd magnet for 1 min and remove the supernatant. 6. Repeat step 4 and 5 once more.
2	 **Loading antibody**	7. Load about 1-10 µg of antibody in 200 µl of Binding & Washing buffer onto the pre-equilibrated magnetic nanobeads. 8. Incubate in a rotator for 10 min at room temperature. 9. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** Supernatant from this step is the **Unbound sample** for checking the binding efficiency.
3	 **Washing magnetic nanobeads**	10. Add 250 µl of Binding & Washing buffer and wash the magnetic nanobeads by gently pipetting. 11. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** Supernatant from this step is the **Washing sample** for checking the washing conditions. 12. Repeat step 10 and 11 once more. *** Note:** After the final wash, the remaining Binding & Washing buffer should be removed completely.
4	 **Antigen and antibody Binding**	13. Add 100-1,000 µl of sample containing target proteins and gently vortex. 14. Incubate in a rotator for 1 hr at room temperature. *** Note:** Make sure that the magnetic nanobeads are resuspended well. 15. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** Supernatant from this step is the **Binding sample** for checking the binding of antigen and antibody.
5	 **Washing magnetic nanobeads**	16. Add 200 µl of Binding & Washing buffer and wash the magnetic nanobeads by gently pipetting. 17. Place the tube on the Nd magnet for 1 min and remove the supernatant. 18. Repeat step 16 and 17 two times. 19. Add 200 µl of Binding & Washing buffer. 20. Resuspend well and transfer it to a new tube. 21. Centrifuge for 1-3 sec at 1,000-3,000 rpm briefly. 22. Place the tube on the Nd magnet for 1 min and remove the supernatant. *** Note:** The Remaining Binding & Washing buffer should be removed completely.

6

Elution

Denaturing elution

23. Add 10 µl of Elution buffer and 10 µl of distilled water (D.W.) and gently mix.
24. Incubate at 70˚C for 10 min.
25. Place the tube on the Nd magnet for 1 min and transfer the elution fraction to a new tube.

*** Note:** Elution fraction from this step is the **Elution sample** for checking the target proteins.

26. Analyze the samples with SDS-PAGE.

Non-denaturing elution

23. Add 20 µl of Elution buffer and gently mix.
24. Incubate for 2 min at room temperature.
25. Place the tube on the Nd magnet for 1 min and transfer the elution fraction to a new tube.
26. Add 2 µl (10% of eluate‡) of Neutralization buffer† to the elution fraction.

*** Note:** Elution fraction from this step is the **Elution sample** for checking the target proteins.

† User may utilize other buffers for neutralization depending on the purpose of the experiment.

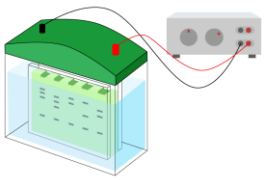
‡ Elution buffer and Neutralization buffer should be mixed at a ratio of 9:1. The pH of mixture should be between 7-8. This only applies when using Elution buffer and Neutralization buffer provided in the kit.

27. Analyze the samples with SDS-PAGE.

Option

- You can also use BIONEER's *MagListo*™-2 Magnetic Separation Rack (Cat. No. TM-1010) instead of a Nd magnet. Refer to the Manual of this product for additional information.

Indentification of samples



Analysis with SDS-PAGE

Analyze the samples with SDS-PAGE.

ex) Protocol for SDS-PAGE.

	Unbound/Washing /Binding	Elution
Sample	5 µl	15 µl
4X Loading dye	5 µl	5 µl
Distilled water (D.W.)	10 µl	-
Total volume	20 µl	20 µl

- Denaturize at 95°C for 5 min.
- Load 5 µl each of "Unbound, Washing and Binding sample" and 10 µl of "Elution sample" on the SDS-PAGE gel.
- Run SDS-PAGE.
- Perform staining with Coomassie Blue R-250.

Using *ExiProgen*™ instrument for automated protein purification

1

Preparation of Cartridge

1. Using a 6-hole punch, place holes onto the corresponding position in Cartridge ① and ② sealing films, included in Cat. No. KA-3001 according to the number of samples (up to 16 wells), and deliver reagents of the *MagListo*™ Kit into corresponding wells stated in figure 1.

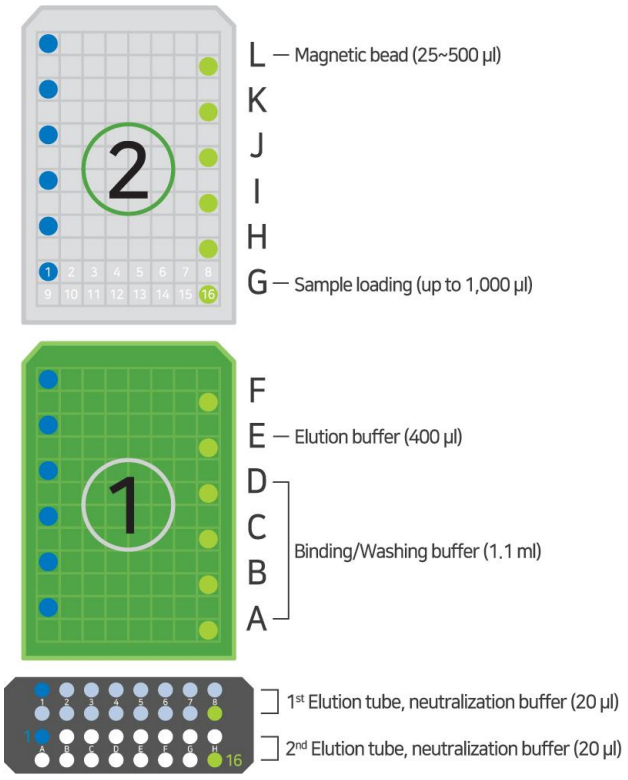


Figure 1. Position of reagents, tubes, and starting material in Cartridges/Elution tube rack.

2

Setting up *ExiProgen*™

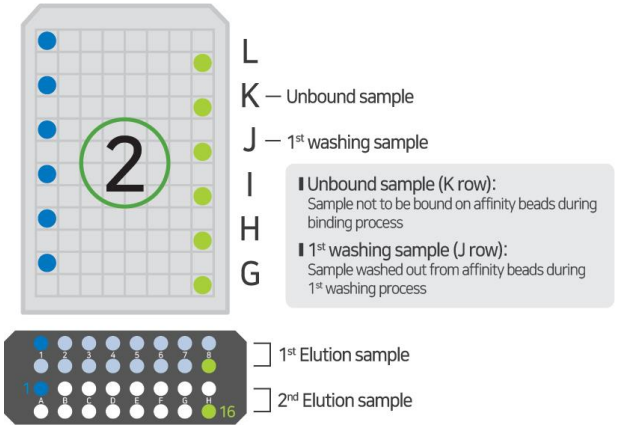
2. Place Cartridge ①, ②, Waste tray, Reaction block, and Disposable tip rack into Base plate.
3. Push the Base plate slowly all the way in, and turn on the *ExiProgen*™.
4. After completion of the setup, run the *ExiProgen*™ instrument as follows.

*** Note:** Updated *ExiProgen*™ protocol ver. 4.3 (or higher) required to run this protocol.

3

Running *ExiProgen*™

5. Turn on the *ExiProgen*™ and press the “Press to start” button in the center of the screen. Soon the *ExiProgen*™ screen with a moving scroll bar will appear, and after a short period of time it moves to the “MENU” screen. The short pause is for the initialization process of the instrument. If it does not move to the next screen, turn off the instrument and call the customer center for A/S.

		6. In the MENU screen, press the “Start” button to select a proper protocol.
		7. In the PREP SETUP screen, input the protocol number **‘342’** Ensure that the following statement appears on the screen. Then select “Enter”. **Prep type: Purification** **Sample SRC: Protein A**
		8. Ensure that everything is correctly installed following the CHECK LIST, then choose “ok”
	  	9. In the Running Mode screen shown on the left, ensure that the statement appears on the screen. Then press “RUN” to initiate protein dialysis. **Prep type: Purification** **Sample SRC: Protein A** 10. “Work Completion” screen appears when the protocol is completed. Open the door and collect final proteins from the dialysis tubes. Remove all components used in the experiment, and choose 1, 2, or ok. *** Note:** UV lamp will be on automatically if the “ok” button is selected to finish the use of *ExiProgen*™.
4	 **Collecting samples**	11. After the "Work Completion" window is displayed, collect the 1st and 2nd elution tubes. Collection of contents of unbound fraction (K row) and/or 1st wash fraction (J row) in Cartridge ② is optional. 

Maintenance

- Reaction block and Waste tray: After washing with water, swap with 70% ethanol and rinse with sterile distilled water.
- Disposable tip rack in *ExiProgen*™: To remove any dirt on the Disposable tip rack, cleanse with 70% ethanol.
- Cartridge: The cartridge with unused wells should be covered with their lid and stored at room temperature.