AccuNanoBead™ Protein A Magnetic Nanobeads (V5/2021-09-27)

[Cat. No.] TA-1022-1, TA-1022-5, TA-1022-10

Introduction

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. This product 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	TA-1022-1	TA-1022-5	TA-1022-10
AccuNanoBead™ Protein A Magnetic NanoBeads	40 mg/ml x 1 ea	40 mg/ml x 5 ea	40 mg/ml x 10 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.

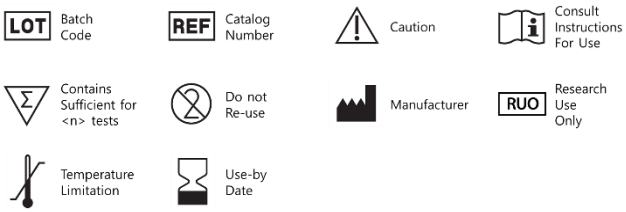
Ordering Information

Description	Cat. No.
AccuNanoBead™ Protein A Magnetic NanoBeads	TA-1022-1 TA-1022-5 TA-1022-10

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.

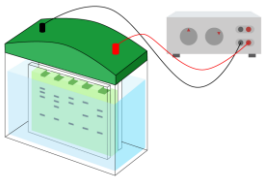
Explanation of Symbols



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.

<table>
<tr><td rowspan="3">6</td><td colspan="2">Elution</td></tr>
<tr><td> 
Denaturing elution</td><td>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 <u>Elution sample</u> for checking the target proteins.
26. Analyze the samples with SDS-PAGE.</td></tr>
<tr><td> 
Non-denaturing elution</td><td>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 <u>Elution sample</u> 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.</td></tr>
<tr><td></td><td> 
Option</td><td>• 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.</td></tr>
<tr><td colspan="3">Indentification of samples</td></tr>
<tr><td></td><td> 
Analysis with SDS-PAGE</td><td>Analyze the samples with SDS-PAGE.
ex) Protocol for SDS-PAGE.
<table><tr><th></th><th>Unbound/Washing /Binding</th><th>Elution</th></tr><tr><td>Sample</td><td>5 µl</td><td>15 µl</td></tr><tr><td>4X Loading dye</td><td>5 µl</td><td>5 µl</td></tr><tr><td>Distilled water (D.W.)</td><td>10 µl</td><td>-</td></tr><tr><td>Total volume</td><td>20 µl</td><td>20 µl</td></tr></table>- 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.</td></tr>
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