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

[Cat. No.] TA-1021-1, TA-1021-5, TA-1021-10

Introduction

AccuNanoBead™ Protein G Magnetic NanoBeads are silica beads conjugated with high purity (>95%) Protein G, which allow specific recognition of antibodies and purification of 1.2 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-1021-1	TA-1021-5	TA-1021-10
AccuNanoBead™ Protein G 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 G Magnetic NanoBeads	
Composition	Silica based magnetic nanobeads
Binding capacity	> 1.2 mg of human IgG/ml of beads
Size	Average 400 nm
Concentration	40 mg/ml

Storage Buffer

AccuNanoBead™ Protein G 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 G 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.

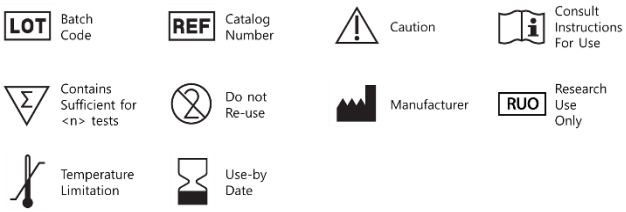
Ordering Information

Description	Cat. No.
AccuNanoBead™ Protein G Magnetic NanoBeads	TA-1021-1 TA-1021-5 TA-1021-10

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



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Experimental Procedures

Steps		Procedure Details
Antibody purification		
1	 **Equilibrating magnetic nanobeads**	1. Resuspend *AccuNanoBead*™ Protein G 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.

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Immunoprecipitation		
1	 **Equilibrating magnetic nanobeads**	1. Resuspend *AccuNanoBead*™ Protein G 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.

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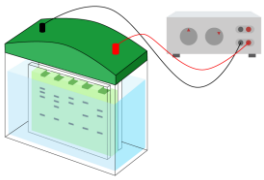
<table>
<tr><td rowspan="3">6</td><td colspan="2">Elution</td></tr>
<tr><td> 
Denaturing elution</td><td>23. Add 10 µl of Elution buffer and 10 µl of distilled water (D.W.) and gently mix.
24. Incubate at 70˚C for 10 min.
25. Place the tube on the Nd magnet for 1 min and transfer the elution fraction 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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