CREATIVE BIOMART®
Assay Kit

Protein G Kit

Product Information

Cat

Kit-0888

Common Name

Protein

Cat.No.

Kit-0888

Product Overview

Protein G Kit includes silica based magnetic nanobeads conjugated with highly purified Protein G (Purity >95%) and buffers required for protein purification. Protein G Kit allows simple and quick experiment due to a fast magnetic response rate. Protein G Kit is not only for antibody purification, but for other immunoprecipitation purpose, such as antigen purification, proteinprotein interaction, and cell separation.

Storage

Protein G Magnetic NanoBeads are supplied as a 4% (w/v) suspension in storage buffer and should be stored at 2~8°C.

Kit Components

Protein G Magnetic NanoBeads 1 mL X 1 ea(4%);
Binding & Washing buffer 20 mL x 2 ea;
Elution Buffer 1 mL X 1 ea;
Neutralization Buffer 1 mL X 1 ea;
Manual 1 ea.
Protein G Magnetic NanoBeads contain 40 mg Beads/mL in storage buffer (phosphate buffered saline, pH 7.4, 0.02% Tween-20, and 0.1% NaN3).

Features & Benefits

Fast Binding: Powerful magnetism reduces experiment time and increases yield.

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Large Surface Area: Average diameter of 400nm provides large surface and allows high binding capacity.
Specificity: Globular beads reduce non-specific binding.

Assay Protocol

1. Immunoglobulin purification
Preparation of Protein G Magnetic Nanobeads
1. Resuspend Protein G Magnetic NanoBeads by gently vortexing the tube.
2. Transfer 200 μL of Protein G Magnetic NanoBeads into a 1.5 mL tube.
3. Place the tube on a magnet to pull down the beads and remove supernatant.
4. Add 1 mL of Binding & Washing buffer and wash the beads.
5. Place the tube on a magnet and remove supernatant.
Binding Immunoglobulin
1. Add 500 μL of sample, and 500 μL of Binding & Washing buffer to the tube containing the beads. Incubate in a rotator for 1 hour at room temperature.
Note) Make sure that the beads are resuspended well. This is important for efficient purification.
2. Place the tube on a magnet and collect supernatant.
Note) Keep supernatant for SDS-PAGE to check binding.
3. Add 500 μL of Binding & Washing buffer to the tube and mix gently to wash the beads. Place the tube on a magnet and remove supernatant. Repeat this process two times more.
Elution
1. Add 100 μL of Elution buffer to the tube and mix well by pipetting. Incubate for 1 minute.
2. Place the tube on a magnet and transfer elute to a new tube. Repeat this process one more.
Note) For better yield, repeat the elution step one more or increase elution buffer volume.
3. Add 10 μL (10% of elute) of Neutralization buffer.

2. Immunoprecipitation
This protocol offers a general guideline for immunoprecipitation. Optimization might be required for your research.
Preparation of Protein G Manetic Nanobeads
1. Resuspend AccuNanoBead™ Protein G Magnetic NanoBeads by gently vortexing the tube.

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2. Transfer 50 μL of AccuNanoBead™ Protein G Magnetic Nanobeads into a 1.5 mL tube.
3. Place the tube on a magnet and remove supernatant.
4. Add 250 μL of Binding & Washing buffer and wash the beads.
5. Place the tube on a magnet and remove supernatant.

Binding Antibody & Antigen

1. Add 1~10 μg antibody in final volume of 200 μL solution to the tube containing the beads and resuspend it well.
2. Incubate in a rotator for 10 minutes at room temperature.
3. Place the tube on a magnet and remove supernatant.
4. Add 250 μL of Binding & Washing buffer to the tube and wash the beads. Place the tube on a magnet to remove supernatant. Repeat this process two times more.
5. Add reagent containing antigen (100~1,000 μL) and mix with beads well.
6. Incubate in a rotator for 1 hour at room temperature.

Note) Resuspend the beads well.

7. Place the tube on a magnet and collect supernatant.

Note) Keep the supernatant for SDS-PAGE to check binding.

8. Add 200 μL of Binding & Washing buffer to the tube containing the beads and mix gently to wash the beads. Place the tube on a magnet and remove supernatant.

Repeat this process two times more.

9. Add 200 μL of Binding & Washing buffer and resuspend well. Transfer it to a new tube.
10. Centrifuge the tube containing beads at 1,000~3,000 rpm for 1~3 sec, briefly.
11. Place the tube on a magnet and remove Binding & Washing buffer as much as possible.

Elution

A. Denaturing elution

1. Add 20 μL of Elution buffer to the tube and mix well by pipetting.
2. Incubate at 70□ for 10 minutes.
3. Place the tube on a magnet and transfer elute to a new a tube.
4. Add reducing agent containing SDS-PAGE sample buffer and mix well. Load the sample to SDS-PAGE.

B. Non-denaturing elution

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1. Add 20 μL of elution buffer to the tube and mix well by pipetting.
2. Incubate for 2 minutes. Place the tube on a magnet and transfer elute to a new tube.
3. Add 2 μL (10% of elute) of Neutralization buffer.

Note) User may utilize other buffer for neutralization depending on the objective of experiment.

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