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Immunomagnetic Labeling and Separation of Escherichia coli Using Protein A-Coated Magnetic Nanoparticles V.2

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We use this protocol and it's working

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Disclaimer

Abstract

This protocol outlines a method for the immunomagnetic labeling and separation of *Escherichia coli* ATCC 25922 using Protein A-coated magnetic nanoparticles (MNPs). The procedure involves culturing *E. coli*, preparing a bacterial suspension, antibody labeling, and subsequent capture using MNPs. The efficiency of bacterial capture is assessed by plate counting, providing a quantitative measure of the labeling process. This method is suitable for applications requiring the isolation of labeled bacterial populations for downstream analyses.

Guidelines

- Ensure all reagents and materials are prepared and available before starting the protocol.
- Maintain sterile conditions throughout the procedure to prevent contamination.
- Perform all centrifugation steps at the specified speed and duration to ensure consistency.
- Use appropriate personal protective equipment (PPE) and adhere to laboratory safety protocols.
- Include negative controls lacking either the antibody or MNPs to assess non-specific binding.
- Labeled bacterial suspensions can be stored at 4°C for up to 24 hours without significant loss of functionality.

Materials

- *E. coli* ATCC 25922 strain
- Plate Count Agar (PCA)
- Phosphate-Buffered Saline (PBS)
- Triton™ X-100 (0.01% solution)
- Rabbit anti-*E. coli* IgG antibody (e.g., ab137967, Abcam)
- Protein A-coated magnetic nanoparticles (BNF-Dextran, Micromod)
- Skimmed milk powder (for blocking solution)
- DynaMag™-2 Magnet (Invitrogen™)
- Spectrophotometer (cuvette-based)
- Centrifuge capable of 3,000 × g
- Rotary agitator
- Sterile microcentrifuge tubes
- Sterile pipette tips

Safety warnings

- Handle all bacterial cultures and reagents following biosafety level 2 (BSL-2) guidelines.
- Dispose of biological waste according to institutional and governmental regulations.
- Use appropriate PPE, including lab coats, gloves, and eye protection.
- Triton™ X-100 is an irritant; handle with care and avoid inhalation or contact with skin and eyes.

Before start

- Thaw frozen *E. coli* ATCC 25922 stock on ice.
- Prepare PBS with 0.01% Triton™ X-100.
- Prepare 0.5% skimmed milk blocking solution in PBS.
- Equilibrate all reagents to room temperature unless otherwise specified.

Bacterial Culture

1 Streak *E. coli* ATCC 25922 from frozen stock onto a PCA plate.

2 Incubate at 37°C for 24 hours.

Preparation of Bacterial Suspension

3 Select a single colony and suspend in 1 mL PBS.

4 Using a cuvette-based spectrophotometer, adjust the optical density at 600 nm to 0.5 to achieve a bacterial concentration of ~1 × 10^8 CFU/mL.

Bacterial Washing Steps

5 Centrifuge at 3,000 × g for 6 minutes.

6 Discard the supernatant and resuspend the pellet in 1 mL PBS with 0.01% Triton™ X-100.

7 Repeat the wash step twice.

8 Finally, resuspend the pellet in 200 µL PBS with 0.01% Triton™ X-100.

Antibody Labeling

9 Add 2 µL of rabbit anti-*E. coli* IgG antibody (4 mg/mL) to the bacterial suspension.

10 Incubate at room temperature for 30 minutes on a rotary agitator.

11 Centrifuge at 3,000 × g for 3 minutes.

12 Discard the supernatant and wash the pellet twice with 1 mL PBS containing 0.01% Triton™ X-100.

13 Resuspend the final pellet in 100 µL PBS with 0.01% Triton™ X-100.

Preparation of Magnetic Nanoparticles

14 Pipette the necesary volume of 100 nm diameter Protein A-coated MNPs into 500 µL PBS with 0.01% Triton™ X-100 to achieve a ratio of 300 MNP per bacterial cell.

15 Place the mixture in a magnetic concentrator for 2 minutes.

16 Discard the supernatant and resuspend the particles in 500 µL PBS with 0.01% Triton™ X-100.

17 Repeat the wash step twice.

18 After the final wash, remove the supernatant and take the tubes off the magnetic separator.

Conjugation of Bacteria with MNPs

19 Add the 100 µL antibody-labeled *E. coli* suspension to the prepared MNPs.

20 Incubate at room temperature for 30 minutes on a rotary agitator.

Blocking and Final Washing

21 Centrifuge the mixture at 3,000 × g for 3 minutes.

22 Discard the supernatant and resuspend the pellet in 1 mL of 0.5% skimmed milk blocking solution.

23 Incubate at room temperature for 10 minutes on a rotary agitator.

24 Centrifuge at 3,000 × g for 3 minutes.

25 Resuspend the pellet in 1 mL PBS with 0.01% Triton™ X-100.

26 Place the tube in a magnetic concentrator for 10 minutes.

27 Carefully pipette the supernatant (containing non-labeled bacteria) into a separate microcentrifuge tube.

28 Resuspend the pellet (containing labeled bacteria) in 1 mL PBS with 0.01% Triton™ X-100.

Control Experiments

29 Prepare negative controls by omitting either the antibody or the MNPs during the labeling process to assess non-specific binding.

Assessment of Capture Efficiency

30 Perform plate counts on both the supernatant and pellet fractions to determine the number of non-labeled (N_F) and labeled (N_L) bacterial cells, respectively.

31 Calculate the capture efficiency (CE) using the formula:

$$CE\ (\%) = N_L / (N_L + N_F) \times 100$$

Storage

32 Store the labeled bacterial suspensions at 4°C.

33 Use within 24 hours to ensure optimal functionality.