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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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## 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  $\sim 1 \times 10^8$  CFU/mL.

## Bacterial Washing Steps

5 Centrifuge at  $3,000 \times g$  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 necessary 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.