



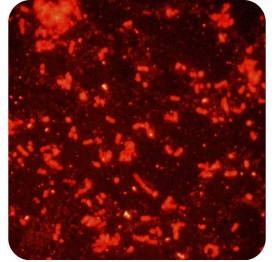
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Version 3

Immunofluorescence Staining of Bacterial Samples V.3

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We use this protocol and it's working

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Abstract

This protocol describes the preparation, fixation and immunostaining of bacterial samples for fluorescence microscopy. It includes detailed steps for monolayer preparation, blocking, primary and secondary antibody incubations, and mounting, with options to optimize slide preparation and antibody incubation for improved reproducibility and signal quality.

Guidelines

- Always keep fluorescent antibodies and stained slides protected from light.
- Keep antibodies on ice during preparation.
- Use clean, particle-free solutions and sterile pipette tips.
- For reproducibility, prefer pre-coated Poly-L-lysine slides when possible.
- Pre-test antibody concentrations if using new lots or targets.

Materials

- 70% Ethanol
- Poly-L-lysine solution (1 mg/mL) or Pre-coated Poly-L-lysine slides
- Phosphate Buffered Saline (PBS), pH 7.4
- PBS with 0.5% Triton X-100 (PBS-Tx)
- Fish Skin Gelatin 0.2% PBS solution (blocking agent)
- Ultrapure water (preferably Milli-Q)
- Primary antibodies (1:100 working solution; on ice)
- Fluorescent secondary antibodies (1:100 working solution; on ice, protected from light)
- 1.5 mL Eppendorf tubes
- Microscope slides
- P200 micropipette and sterile tips
- Humid chamber
- Slide holders for washing
- DAKO pen
- Filter paper
- Coverslips
- Mounting media (e.g., VectaShield® or Fluoromount™)
- Clear nail varnish (optional)

Safety warnings

- ! ▪ Handle all fluorescent reagents in low light to prevent signal degradation.
- Avoid letting slides dry during staining steps.
- Ensure thorough but gentle washes to prevent detaching bacterial monolayers.
- Carefully apply and seal coverslips to prevent sample loss and preserve fluorescence.

Before start

- Turn on the humid chamber to equilibrate at desired temperature.
- Prepare all antibody working solutions fresh.
- Clean glassware and ensure the workspace is free of dust and particles.
- Have all washing buffers (PBS, PBS-Tx) ready and at room temperature.

Bacterial Preparation

- 1 Transfer 1 mL of 24-hour bacterial culture into a 1.5 mL Eppendorf tube.
- 2 Centrifuge at $4000 \times g$ for 4 minutes.
- 3 Discard supernatant and resuspend pellet in 1 mL PBS.
- 4 Repeat centrifugation and resuspension two more times (total three washes).
- 5 After final wash, resuspend pellet in 100 μ L PBS.

Slide Preparation

6 **Option 1: Manual Poly-L-lysine Coating**

- 6.1 Clean slides with 70% ethanol.
- 6.2 Pipette 100 μ L Poly-L-lysine solution onto filter paper.
- 6.3 Firmly swipe the filter paper once across each slide.

7 **Option 2: Pre-coated Slides**

- 7.1 Use pre-coated Poly-L-lysine slides directly.
- 8 Draw hydrophobic barriers using the DAKO pen.



- 9 Pipette 100 μ L bacterial suspension into compartments.
- 10 Incubate in a humid chamber at room temperature for 10–15 minutes.
- 11 Gently blot excess with a paper towel.
- 12 Check under microscope for monolayer formation.

Blocking

- 13 Pipette 100 μ L 0.2% Fish Skin Gelatin into each compartment.
- 14 Incubate in a humid chamber for 30 minutes at room temperature.
- 15 Wash slides:
 - Brief rinse with PBS-Tx
 - 5-minute wash with PBS

Primary Antibody Incubation

- 16 Pipette 30 μ L primary antibody working solution into experimental compartments.
- 17 Pipette 30 μ L Fish Skin Gelatin into control compartments.
- 18 Incubation options:
 - 1 hour at 37°C in humid chamber, or
 - Overnight at 4°C in humid chamber (recommended for improved sensitivity)

- 19 Wash slides:
- Brief rinse with PBS-Tx
 - 10-minute PBS-Tx wash at room temperature
 - 5-minute PBS wash

Secondary Antibody Incubation

- 20 Add 30 μ L fluorescent secondary antibody to all compartments.
- 21 Incubate for 30 minutes at 37°C in the dark.
- 22 Wash slides:
- Brief rinse with PBS-Tx
 - 10-minute PBS-Tx wash at room temperature
 - 5-minute PBS wash

Mounting

- 23 Apply mounting medium (e.g., VectaShield® or Fluoromount™).
- 24 Gently place coverslip, avoiding air bubbles.
- 25 Optionally seal coverslip edges with clear nail varnish.
- 26 Store slides in the dark until imaging.
- 27 Visualize using a fluorescence microscope.



Protocol references

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