

Sep 19, 2025

Transmission Electron Microscopy (TEM) Protocol for *Pseudomonas* sp.

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn4bmqv5d/v1>

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Protocol Citation: Md Sahadat Ali, Fatima Tuz Zohora Mony, Jonathan D. Eisenback 2025. Transmission Electron Microscopy (TEM) Protocol for *Pseudomonas* sp.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.14egn4bmqv5d/v1>

Manuscript citation:

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Protocol status: Working

We use this protocol and it's working

Created: March 20, 2025

Last Modified: September 19, 2025

Protocol Integer ID: 124699

Keywords: Transmission Electron Microscopy (TEM), Bacterial ultrastructure, Pseudomonas, Glutaraldehyde fixation, Negative staining, Flagella visualization, protocol for pseudomonas sp, resolution visualization of bacterial morphology, pseudomonas sp, transmission electron microscopy, pseudomona, bacterial morphology, electron microscopy, flagellar structure

Disclaimer

This protocol has been optimized for the TEM analysis of *Pseudomonas* sp. Researchers must follow biosafety regulations when handling bacterial cultures and adhere to institutional guidelines for electron microscopy sample preparation. The authors are not responsible for procedural errors or misuse.

Abstract

This protocol describes the preparation, fixation, staining, and imaging of *Pseudomonas* sp. using Transmission Electron Microscopy (TEM). The method provides high-resolution visualization of bacterial morphology and flagellar structures, confirming motility-related features. Proper fixation and staining ensure cellular integrity and contrast for high-quality micrographs

Guidelines

- Ensure all sample preparation steps are performed under sterile conditions to avoid contamination.
- Use appropriate PPE (gloves, lab coat, and safety glasses) when handling glutaraldehyde and UranylLess stain.
- Avoid excessive drying of the bacterial suspension before staining to preserve cellular integrity.
- Handle TEM grids carefully using fine-point forceps to prevent structural damage.
- Ensure the microscope is properly calibrated before imaging to obtain high-resolution micrographs.

Materials

Reagents & Solutions

- LB Lite Broth – For bacterial culture growth
- Double-Deionized (DDI) Water – For washing steps
- 4% Glutaraldehyde in 0.1M Sodium Cacodylate Buffer (pH 7.4) – For fixation
- UranyLess Stain – Electron-dense contrast enhancer for TEM

Consumables & Equipment

- 120 kV FEI T12 Tecnai Spirit Transmission Electron Microscope (TEM)
- Formvar-Coated Copper Grids – For sample preparation
- Micropipettes & Filtered Tips – For precise liquid handling
- Centrifuge – For bacterial cell collection
- Fine-Point Forceps – For grid handling
- Grid Storage Box – To protect prepared grids before imaging

Safety warnings

- !
 - Glutaraldehyde is toxic and should be handled under a fume hood with proper PPE.
 - UranyLess stain is a heavy metal-based solution; proper disposal procedures must be followed.
 - Avoid direct contact with TEM grids, as fingerprints and static can interfere with imaging.
 - Ensure all biohazardous waste (bacterial cultures and used grids) is disposed of according to institutional guidelines.

Ethics statement

This protocol involves the Transmission Electron Microscopy (TEM) analysis of a plant growth-promoting bacterial species (*Pseudomonas* sp.). No animals or human subjects were involved in this study. However, researchers must adhere to institutional biosafety regulations when handling microbial cultures, fixation chemicals (such as glutaraldehyde), and heavy metal-based stains.

If this protocol is adapted for studies involving animal or human samples, prior approval must be obtained from an Institutional Animal Care and Use Committee (IACUC) or an equivalent ethics committee. Researchers must comply with internationally accepted ethical standards, and any relevant permit numbers and committee approval should be documented accordingly.

Proper biosafety precautions, chemical handling protocols, and ethical considerations must be followed to ensure responsible research practices.

Before start

- Prepare all reagents and solutions in advance.
- Label sample tubes and TEM grids properly.
- Ensure the TEM microscope is ready for imaging with proper alignment.
- Work in a clean and contamination-free workspace.

Procedures

1 Bacterial Culture Preparation

1. Grow *Pseudomonas* sp. in LB Lite Broth under optimal conditions for 24 hours.
2. Harvest bacterial cells by centrifugation at an appropriate speed (e.g., 10,000 x g for 5 minutes).
3. Carefully discard the LB medium, retaining only the bacterial pellet.

Note: Fresh cultures yield the best results for TEM imaging.

2 Washing and Fixation

1. Wash the bacterial pellet twice with DDI water to remove residual media.
2. Fix bacterial cells overnight in 4% glutaraldehyde prepared in 0.1M Sodium Cacodylate Buffer (pH 7.4) at 4°C.

Note: Fixation preserves the bacterial morphology for high-resolution imaging.

3 Sample Preparation for TEM

1. Pipette 20 µL of bacterial suspension onto a formvar-coated copper grid.
2. Allow the bacterial sample to adhere for 30 seconds.
3. Carefully remove excess liquid using filter paper or fine-point forceps.
4. Apply 20 µL of UranylLess stain to the grid for 60 seconds to enhance contrast.
5. Remove excess stain and allow the grid to air dry before imaging.

Note: Ensure gentle handling to avoid cell damage during staining and drying.

4 Imaging and Analysis

1. Examine the prepared bacterial sample using a 120 kV FEI T12 Tecnai Spirit TEM.
2. Capture high-resolution micrographs to visualize:
 - Overall cell morphology
 - Flagellar structure (monotrichous flagellum)
3. Analyze images to confirm motility-related structures and bacterial surface features.

Note: Adjust TEM settings for optimal contrast and resolution.

5 Expected Results

- High-resolution TEM micrographs showing detailed bacterial morphology and flagellar structure.
- Well-preserved cellular integrity due to proper fixation and staining.
- No significant sample aggregation or damage, ensuring accurate visualization.

Protocol references

Mrazova K, Bacovsky J, Sedrlova Z, Slaninova E, Obruca S, Fritz I, et al. Uranyl-Less Low Voltage Transmission Electron Microscopy: A Powerful Tool for Ultrastructural Studying of Cyanobacterial Cells. *Microorganisms*. 2023 Mar 29;11(4):888.