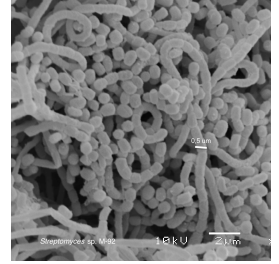


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🌐 Scanning electron microscopy protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkk796l5r/v1>



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Rene Flores Clavo: CENTRO DE INVESTIGACIÓN E INNOVACIÓN EN CIENCIAS ACTIVAS MULTIDISCIPLINARIAS

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Protocol Electronic Micr...



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Protocol status: Working

We use this protocol and it's working

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Keywords: Scanning electron, microscopy protocol, bacterial identification's, LBME, CIICAM, scanning electron microscopy processing, electron microscopy protocol, electron microscopy processing, electron microscopy, scanning electron, microscopy, critical point chamber, sputter coating, acquisition of image, fixation

Abstract

This protocol briefly summarizes the basic steps of a scanning electron microscopy processing. The methods adopted for fixation, post-fixation, dehydration, drying in a critical point chamber, sputter coating, and the visualization and acquisition of images are described here.

Materials

1. Sample;
2. Glutaraldehyde, 2,5%;
3. Sodium cacodylate buffer (pH 7.3) 0.1 M;
4. Osmium tetroxide, 1.0%;
5. Acetone;
6. CO₂;
7. Aluminum stubs;
8. Balzers CPD-030 Critical Point Dryer;
9. Balzers SCD 050 Sputter-Coater;
10. Scanning Electron Microscope JEOL JSM 5800LV, at 10 kV;
11. SemAfore 5.21 software.

Protocol materials

⊗ Glutaraldehyde 25% Aqueous Solution 10 × 10 ml ampoules **Electron Microscopy Sciences Catalog #16220**

⊗ Sodium cacodylate trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0250**



MATERIAL SELECTION

- 1 1 mm² samples were selected in the colony.

FIXATION

4h

- 2 Samples were fixed in a solution of [M] 2.5 % volume



Glutaraldehyde 25% Aqueous Solution 10 × 10 ml ampoules **Electron Microscopy Sciences Catalog #16220**

and [M] 0.1 Mass Percent



Sodium cacodylate trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0250**

buffer (pH 7.3), at Room temperature, for 04:00:00.

4h

WASHING AFTER FIXATION

- 3 Samples was then rinsed three times in [M] 0.1 Mass Percent



Sodium cacodylate trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0250**

(pH 7.3), at Room temperature, for 00:20:00 for each rinse.

20m

POST-FIXATION

- 4 Samples was then post-fixed with [M] 1 % volume osmium tetroxide in

[M] 0.1 Mass Percent



Sodium cacodylate trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0250**

buffer, (pH 7.3), at Room temperature, for 01:00:00, protected from ambient light.

1h

WASHING AFTER POST-FIXATION



5 Samples was then rinsed three times in sodium [M] 0.1 Mass Percent

20m



Sodium cacodylate trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0250**

buffer 7.3 , at Room temperature , for 00:20:00 for each rinse.

DEHYDRATION

6 Samples were dehydrated with ascending acetone series, for 00:20:00 minutes

20m

each step - [M] 30 % volume ; [M] 50 % volume , [M] 70 % volume ,
[M] 90 % volume , [M] 100 % volume (last one step, for three times).

CRITICAL POINT DRYING

7 Samples was then dried using the critical point method with CO₂.

MOUNTING ON THE STUBS

8 Samples was then placed on aluminum stubs.

SPUTTER-COATER

9 Samples was then coated with a layer of 30–40 nm gold using a Balzers SCD 050 sputter-coater.

OBSERVATIONS AND IMAGE ACQUISITION

10 Observations and photomicrograph acquisitions were obtained using a JEOL JSM 5800LV at 10 kV with SemAfore 5.21 software.