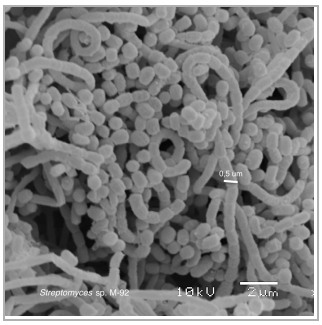

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Scanning electron microscopy protocol

DOI
dx.doi.org/10.17504/protocols.io.j8nlkk796l5r/v1

Rene Flores Clavo[1], Francisco Breno S. Teófilo[1]

[1]Universidade Estadual de Campinas

Rene Flores Clavo: CENTRO DE INVESTIGACIÓN E INNOVACIÓN EN CIENCIAS ACTIVAS MULTIDISCIPLINARIAS
Francisco Breno S. Teófilo: Electron Microscopy Laboratory - University of Campinas

RENE FLORES | Protocol Electronic Microscopic

Rene Flores Clavo
Universidade Estadual de Campinas, Centro de Investigación e...

COMMENTS 0

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.

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PROTOCOL CITATION

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KEYWORDS

Scanning electron, microscopy protocol, bacterial identification's, LBME, CIICAM

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MATERIALS TEXT

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.

MATERIAL SELECTION

1 1 mm^2 samples were selected in the colony.

FIXATION

4h

4h

2 Samples were fixed in a solution of [M] 2.5 % volume Glutaraldehyde 25% Aqueous Solution 10 x 10 ml ampoules **Electron Microscopy Sciences Catalog #16220** and [M] 0.1 Molarity (M) Sodium cacodylate trihydrate **Merck Millipore Sigma Catalog #C0250** buffer (pH 7.3), at Room temperature, for 04:00:00.

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WASHING AFTER FIXATION

20m

3 Samples was then rinsed three times in [M] 0.1 Molarity (M) Sodium cacodylate trihydrate **Merck Millipore Sigma Catalog #C0250** (pH 7.3), at Room temperature , for 00:20:00 for each rinse.

POST-FIXATION

1h

4 Samples was then post-fixed with [M] 1 % volume osmium tetroxide in [M] 0.1 Molarity (M) Sodium cacodylate trihydrate **Merck Millipore Sigma Catalog #C0250** buffer, pH 7.3 , at Room temperature , for 01:00:00 , protected from ambient light.

WASHING AFTER POST-FIXATION

20m

5 Samples was then rinsed three times in sodium [M] 0.1 Molarity (M) Sodium cacodylate trihydrate **Merck Millipore Sigma Catalog #C0250** buffer pH 7.3 , at Room temperature , for 00:20:00 for each rinse.

DEHYDRATION

20m

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

MOUTING ON THE STUBS

8 Samples was then placed on aluminum stubs.

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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 aquisitions were obtained using a JEOL JSM 5800LV at 10 kV with SemAfore 5.21 software.

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