Jun 14, 2022

Scanning Electron Microscopy - Focused Ion Beam Biological Sample Preparation

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dx.doi.org/10.17504/protocols.io.36wgq42kyvk5/v1

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The combination of the scanning electron microscopy (SEM) with a focused ion beam (FIB), represents a pioneering and interesting tool to allow the investigation of the relationship occurring at the interface between cells and biomaterials. Herein, we provide a suitable protocol for cell-biomaterial sample preparation before imaging by SEM-FIB.

DOI

dx.doi.org/10.17504/protocols.io.36wgq42kyvk5/v1

Ludovica Parisi 2022. Scanning Electron Microscopy - Focused Ion Beam Biological Sample Preparation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq42kyvk5/v1

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Nov 16, 2021

Jun 14, 2022

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FIXATION 40m

1 Remove culturing medium from the samples.

2 Gently wash the samples twice in Phosphate Buffer Saline.

3 Cover the surface of the samples with **Gluthraldehyde 2.5%** in **Na-Cacodylate 0.1M** buffer. 30m

4 Remove the Glutaraldehyde 2.5% in Na-cacodylate 0.1M buffer from the samples and cover the surface of the samples with **Na-Cacodylate 0.1M** buffer. 5m

5 Remove the Na-cacodylate 0.1M buffer from the samples.

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DEHYDRATION 1h

6 De-hydrate the samples in **Ethanol at increasing concentrations (35%, 55%, 70%, 90%, 95%)**. Each alcohol stands for 10 minutes at room temperature. 50m

7 Store the samples in EtOH 95% at 4°C until further processing.

8 Cover the samples with **Ethanol 99%**. 10m

9 Remove The Ethanol 99% from the samples and freeze dry the samples with **Liquid Carbon Dioxide**.

SPUTTER COATING 30s

10 Sputter coat the samples for 30 seconds with Gold using a coating device 30s

SAMPLE OBSERVATION

11 Perform SEM-FIB analysis of the samples. We suggest to perform the SEM analysis at 5keV and to cross-section the samples with a Gallium ion beam accelerated at 30kV.

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