Sample preconditioning before scanning electron microscopy (SEM)

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DISCLAIMER

ABSTRACT

Bacterial samples need to be preconditioned before visualized by scanning electron microscope (SEM). This protocol describes preconditioning methods of bacteria before SEM.

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Bacterial samples preconditioning 10m

1 Obtain a small quantity of bacteria to be tested from a culture.

2 Centrifuge the samples at **5000 rpm, 4°C, 00:10:00** . Resuspend the pellet using 1X phosphate buffed saline (PBS). Repeat this step for three times. Keep the supernatant after the third centrifuge and preserve the samples at **4 °C** . 10m

SEM sample preconditioning 2h

3 Wash silica wafers with ddH_2O and acetone for three times.

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4 Add samples onto the silica wafer using a pipette tip.

5 Incubate the silica wafers in fixation solution (2.5% glutaraldehyde in 1X PBS) at **4 °C** overnight.

6 Incubate the samples in ethanol solution with an increasing concentration (40%, 70%, 96%, 100%) for **01:00:00** each concentration. 1h

7 Dry the samples at room temperature for **01:00:00** . 1h

8 Use a precision etching coating system, sputtering the samples with Au-Pd alloy.

If samples contain gold, sputter the samples with chromium.

9 The samples are ready to be visualized using SEM

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