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Sample preconditioning before scanning electron microscopy (SEM)

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We use this protocol and it's working

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Abstract



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

Troubleshooting



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 buffered 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 ddH₂O and acetone for three times.
- 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.
- 7 Dry the samples at room temperature for  01:00:00 .
- 8 Use a precision etching coating system, sputtering the samples with Au-Pd alloy.

1h

1h

Note

If samples contain gold, sputter the samples with chromium.



9 The samples are ready to be visualized using SEM