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# Sample preconditioning before scanning electron microscopy (SEM)

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1 Works for me

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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.

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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 <sup>10m</sup> phosphate buffed saline (PBS). Repeat this step for three times. Keep the supernatant after the third centrifuge and preserve the samples at  **4 °C** .

## SEM sample preconditioning

2h

- 3 Wash silica wafers with ddH<sub>2</sub>O 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. <sup>1h</sup>
- 7 Dry the samples at room temperature for **01:00:00** . <sup>1h</sup>
- 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