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SEM General Fixation

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Works for me

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Eadewunm

ABSTRACT

General SEM Fixation Procedure

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ABSTRACT

General SEM Fixation Procedure

BEFORE STARTING

Fixative:

One (1) ml. 25% Glutaraldehyde was mixed with 3.2 ml. GD (glass distilled) H₂O – MilliQ H₂O is fine. This gives you 4.2 ml. of 6% Glutaraldehyde.

- Because it is in a 10mL ampule – 10mL Glut + 32 mL H₂O

Mix the 6% Glutaraldehyde and the 0.2M buffer together in a 1:1 mix.

This gives 3% Glutaraldehyde in 0.1M.

The volume of the buffer should be 2-3X volume = follow the suggested volumes at each fixation step.

To order anhydrous ethanol (200 proof), Call Chem Stores (4-3081) to get the most up to date cost of 1 pint. Send Bridgit in the division of biology accounting office an e-mail saying you want ethanol from chem store = bcarpen1@utk.edu. Give her the lab account number and how many pints we want and the cost per pint. She will send a transfer order to your e-mail. You can pick up the ethanol from the bottom of the chemistry building.

General SEM Fixation Procedure

- 1 Grow 50mL cells to appropriate OD600 and wash in 1X PBS. Pour of supernatant.
- 2 Primary Fixation:
 - 3% Glutaraldehyde in 0.1M cacodylate (1X PBS)* = add 500uL and vortex briefly
 - Fix for 1 hour at room temperature.*Any buffer should be fine = use 1X PBS
- 3 Rinse the sample in a buffer (1X PBS) at least 3 times for 10 minutes each.
- 4 Secondary Fixation: (Not usually required)
 - 2% Osmium tetroxide in 0.1M cacodylate (1X PBS) = add 200uL
 - Fix for 1 hour at room temperature.
- 5 Rinse the sample in the water at least 3 times for 10 minutes each.**

**If working with single cells such as bacteria, allow the sample to settle onto a 3X4mm silicon chip after the final water wash. The Si chip (clean off with Methanol – cells go on SHINY side) is then processed through the remaining dehydration steps
- 6 Put the samples at 4°C overnight in 500uL 1X PBS
- 7 Spin down before heading to JIAM. – Don't resuspend
- 8 Bring samples to JIAM – SEM facility
- 9 Clean the silicon chip with methanol
- 10 Poly-L-lysine – add 20uL to the chip and let sit for 2 minutes – blow the liquid off with compressed air
- 11 Add 20uL of cells to the silicone chip and let sit for 25 minutes.

12 Dehydrate sample through a graded ethanol series

25% Ethanol 10 min.

50% Ethanol 10 min.

70% Ethanol 10 min.

95% Ethanol 10 min.

100% Ethanol 10 min

100% Dry Ethanol 10 min – Freshly opened container of anhydrous ethanol

The purpose of 2-100% Ethanol steps is to account for any water that transfers from 95%.

13 Critical point dry – Follow the protocol