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SEM General Fixation

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

General SEM Fixation Procedure

PROTOCOL CITATION

Elizabeth Fozo 2020. SEM General Fixation. **protocols.io** https://protocols.io/view/sem-general-fixation-bqndmva6

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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) H20 – 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% Gultaraldehyde 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^{O}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