Bacteria or Tissue Cultured Cell SEM Sample Preparation

(Tissue Cultured Cells in microfuge tubes)



Procedure for fixation and dehydration of samples for Scanning Electron Microscopy

**Protective Equipment**; Safety glasses, Lab coat, gloves and Chemical Fume hood

Remember to **label all vials** with; sample name, fixative type, your name & date

Fixation: 

2% Paraformaldehyde /2-3% Glutaraldehyde in buffer\* Overnight, 4° C

 and/or

Microwave fix (**optional**) - 2.5 minutes, Power: 250 W, Cut off temp 28° C

 2 % Paraformaldehyde/2 % Glutaraldehyde

 Rest at room temperature 5 minutes

Rinse: With buffer 3x 5 – 10 min ea

Post Fix: 

1 - 2% OsO4 in ddH2O 2 hr, rt or

 overnight at 4**°** C

Rinse: ddH2O 3x 5 min

Dehydration: 

30% Ethanol 10 mins

 50% Ethanol 10 mins

 70% Ethanol 10 mins

 95% Ethanol 10 mins

 100% Ethanol 3x, 10 m ea

100 % HMDS -  2x, 10 m

Final Drying:

* Pipet sample/HMDS mix onto glass coverslip on SEM stub and let air dry in hood
* Or leave sample in vial/tube & pipette out most of the HMDS (leave just enough to cover sample).
* Let air dry in fume hood.