TEM Sample Prep: Cells, Bacteria

Label sample holders with sample no/details
1. Centrifuge (3min @ 3000rpm) cells to get a pellet, discard supernatant
2. Wash serum and media away with buffer (**0.075M phosphate buffer)
   Centrifuge cells to get a pellet
3. Remove buffer

Place samples on the rotator after addition of solutions & between pelleting:

4. Add ***2.5% Glutaraldehyde/Formaldehyde** (**GA/FA**) solution and fix for 30 min
   Centrifuge cells to get a pellet
5. Remove fixative solution
6. Wash with **phosphate buffer 3 times for 10 minutes each wash
   Centrifuge cells to pellet between every step
7. Remove buffer
8. **In the fume cupboard: Danger – do not inhale, prevent skin contact, ensure proper closure of sample holders**
   Add 1% **Osmium Tetroxide (OsO_4)** solution and post-fix for 30 min
   Centrifuge cells to pellet

 # Start to weigh off & mix resin components for later usage

9. Remove & discard **OsO_4** fixative solution in marked waste bottle in **Fume cupboard – add first buffer of step 10 in fume cupboard**
10. Wash with **phosphate buffer 3 times for 10 minutes each wash
    Centrifuge cells to pellet between every step
11. Remove buffer
12. Dehydrate by using a graded series of ethanol (30%, 50%, 70%, 90% and 3x100%) for 10 minutes each - Centrifuge cells to pellet between every step
13. Leave in the last 100% ethanol – 30 minutes
14. Centrifuge cells to get a pellet
    Remove ethanol
15. Add a 50:50 mixture of epoxy resin and 100% ethanol and leave for 1 hour. Centrifuge cells to get a pellet
16. Remove ethanol: resin mixture
17. Add epoxy resin mixture and leave for 1 hour
    Centrifuge cells a pellet
18. Remove the resin
19. Add fresh resin and a sample number and embed sample in molds/Eppendorfs
20. Place in oven for 24 hours polymerization
21. Remove from oven
22. Block Trimming and sectioning

***2.5% GA/FA Fixative:**
1 ml **25%** GA // 0.5 ml **50%** GA
1 ml 25% Formaldehyde
3 ml D H2O
5 ml Buffer // 5.5 ml Buffer

2.5% GA:
1 ml 25% GA
5 ml Buffer
4 ml D H2O

****Washing Buffer:** Dilute 1.5M Phosphate Buffer 1:1 with D H2O