Immunolabelling of thin sections for TEM

Materials

- PBS-T. Dissolve 1PBS tablet in 200ml SDW then add 0.1ml (w/v) Tween 20 to 200ml PBS buffer (0.05% Tween)
- 1% BSA. Dissolve 0.1g BSA (98%) in 10ml PBS-T. Vortex then leave for 10 minutes
- Dilute all antibodies as necessary in PBS-T

Method

- Block on 1% BSA for 30 minutes at room temperature
- Incubate on primary antibody diluted 1:100 in PBS-T for 1 hour at 37^{o}C or overnight at 4^{o}C
- Wash on PBS-T 3 times for 5 minutes each at room temperature
- Block on 1% BSA for 30 minutes at room temperature
- Incubate on secondary antibody diluted 1:50 in PBS-T for 1 hour at 37^{o}C or overnight at 4^{oo}C
- Wash on PBS-T 3 times for 5 minutes each at room temperature
- Wash on SDW twice for 5 minutes each at room temperature
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Notes

- Check which animal primary antibody was raised in before selecting appropriate secondary antibody
- Dilutions listed in the method are a suggested starting point and may need to be adapted for your antibodies
- Do not allow the grids to dry at any time during this procedure
- 20μl drops are sufficient for each grid