



Immunogold labelling for Extracellular vesicle (EVs)

Noted by KJ at 09 Dec. 2020

1. Pre-check EVs concentration:

EVs samples should be checked by negative stain & NTA for checking morphology & concentration. (Over 5×10^{10} EVs/mL)

2. Plasma coating on Ni-carbon grid

3. Cover Ni grid on 5 μ L sample droplet, 10-15 min

4. 1% BSA/PBS wash, 1min X3

5. 1% BSA/PBS as **blocking** buffer for 30 min

6. 1% BSA/PBS + 0.01-0.02% Digitonin as **permeabilization** buffer for 2 min

7. 1% BSA/PBS wash, 1min X3

8. **1st Ab**, 10~20-fold dilution in 1% BSA/PBS, 90 min at RT or 4°C O/N

9. 1% blocking buffer wash, 1min X3

10. **2nd Ab**, 10-fold dilution in 1% BSA/PBS, 60 min at RT

11. 1% BSA/PBS wash, 1min X4

12. PBS wash, 1min X3

13. DDW wash 1min X2

14. 2% UA for **negative stain**, 1 min

15. Air dry