Fixing for Cyro-immunogold EM

0.4 M PHEM Buffer

pipes	**240mM**
hepes	**100mM**
MgCl2	**8mM**
EGTA	**40mM**
pH	**6.9**

Paraformaldehyde(PFA) 4% in 0.2M PHEM (2X PFA)

- make 16% stock from PFA powder
- bring the weighing scale, hot plate, and PFA inside the hood
- heat about 300ml dH20 to 80C
- transfer 0.34g Na2CO3 into a 200ml cylinder put a stirring bar in
- pour about 150ml hot water into the cylinder bring this to hot plate and keep warm while stirring
- transfer 32g of PFA powder into the cylinder
- the PFA will dissolve bring the fluid level up to 200ml

Paraformaldehyde (PFA) 4% + Glutaraldehyde (GA)0.4% in 0.2M PHEM (2X PFA/GA)

- use EM grade glutaraldehyde 8% solution from Polysciences, stored at 4C in a closed box
- make fresh mixture:
  10ml 0.4M PHEM + 5ml PFA 16% + 1ml GA 8% + 4ml H2O

Protocol to fix cells

- provide cells with fresh medium the day before the experiment
- use cell culture directly from incubator; do not wash the cells
- cells are at 37C and fixative is at room temperature or 37
- for cells in suspension add 5ml of 2x fixative to 5ml cell culture (2-5.10^6 cells)
- for cells on a 10cm petri-dish add 5ml 2X fixative to 5ml medium

- fix at room temperature for 1 hr in PFA/GA
- remove fix; add fresh 1x fix drop wise
- fix for 1 hr room temperature
- do not shake during fixation period
- scrape cells in fixative with a rubber policeman
- transfer with Pasteur pipet
- after harvesting; spin for 5 min at 1000rpm
- carefully resuspend pellet in 1ml 0.2%PFA in 0.1M PHEM
- transfer cells with Pasteur pipet into screw-to[tube and ship in suspension