

Acrosome reaction assay

1. Prepare sperm into HTF + 3 mg/mL BSA ☐ Swim-out
2. Leave sperm in capacitation drop for 50 min ☐ aliquot 1:10 for Swim-up ☐ Count
3. Dilute 6×10^6 sperm/mL into a tube (60 min capacitation)
4. Add treatment to test acrosome reaction
5. Incubate for 60 min or at time-point of interest
6. Treat the control with calcium ionophore $10 \mu\text{M}$ for 10 min
7. Fix in 4% paraformaldehyde (in HTF + BSA) for 10 min
8. Spin down at 3,000 g for 3 min
9. Discard supernatant, be careful not to disturb the pellet (very loose)
10. Smear on charge slide
11. Let it dry
12. Stain with Coomassie Blue G-250 in 50% methanol and 10% acetic acid for 2 min
13. Rinse with water
14. Dry completely
15. Coverslip with permount