High pressure freezing and Freeze Substitution of Nematodes

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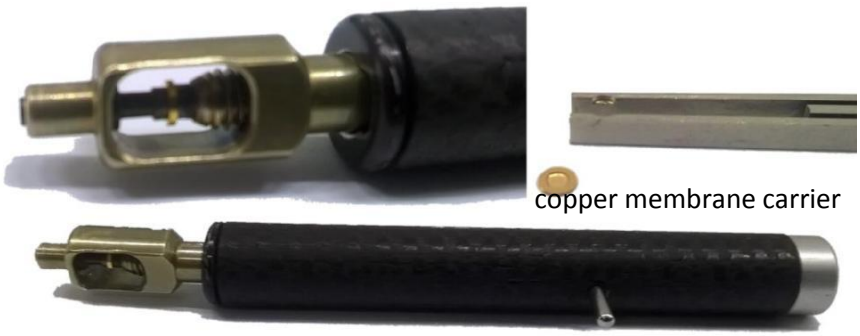
High Pressure Freezing: EMPact (Leica) followed by AFS: Automatic Freeze Substitution (Leica)

EMpact (identical for immunology and morphology)

20% BSA as cryoprotectant, membrane carrier, egg lecithin(20µg/ml)

- Prepare carrier with egg-lecithin
- Fill carrier with BSA 20%
- Transfer (2 or 3) nematodes to the membrane carrier
- EMPact

EMPact Leica



copper membrane carrier

EMPact loading device

AFS: Automatic Freeze Substitution (Leica)

A. AFS cocktail for morphology:

1% OsO4 in 10 ml dried aceton+1% H_2O+0,5% GA

9400 µl dry acetone

0,1g OsO4 (crystal)

100 µl H20

500 µl glutaraldehyde (10 %solution in acetone)

AFS: -90°C 27h

2°C/h (9h)

-60°C 12h

2°C/h (15h)

-30°C 32h

2°C/h (17h)

4°C

Rinse fixation cocktail with dry acetone.

Impregnation with Spurr (Low viscosity embedding medium)

Polymerization for 8h at 70°C

B. AFS: cocktail for immuno

10ml dried aceton+O,1% GA (10% GA in acetone)+2% water

9700 µl dry acetone

100 µl glutaraldehyde (10 % solution in acetone)

200 µl H2O

AFS: -90°C 27h

2°C/h (15h)

-60°C 12h

2°C/h (15h)

-30°C 32h

2°C/h (17h)

4°C

Remove the substitution cocktail :rinsing 3 times with dry aceton and impregnate with LRwhite.

Polymerization in AFS with UV

0°C 24h

2°C/h 10h

20°C 24h

2°C/h 8,5h

37°C 72h ***Leica EM-AFS***

Immunolocalization

localisation of Major Sperm Protein (MSP)

1. 5’ PBS
2. 30min BS (bovine serum 5%; Aurion) Blocking solution
3. 5x5’ washing in IB (incubation buffer):10ml PBS + 100µl BSA-c (10% Aurion)+2 drops HCl (pH 7!)
4. 60 min Ab prim in IB
5. 5x5’ washing in IB
6. 30 min Ab bridge. (RAMs; 1:100) DAKO
7. Washing 5x5’ IB
8. 30 min PAG (protein A 10nmgold)
9. Washing 2x5’ IB
10. 3x5’ PBS

rinse in bidi distilled water (3x)