

EMBEDDING SAMPLES IN PARAFILM

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This protocol assumes you already have the samples stored in 4% formaldehyde.

DAY 1

Sample in 4% formaldehyde
↓
+1L cold **1x PBS***, store on ice in the cold room for **30min**
↓
Transfer sample to 250ml pyrex jar, add 100ml **95% EtOH** and 100ml **1x PBS**
for **1hr 30min**
↓
Transfer to **70% EtOH** (in PBS), cold room, **1hr 30min**
↓
Transfer to **80% EtOH** (in PBS), cold room, **1hr 30min**
↓
Transfer to **90% EtOH** (in PBS), cold room, **1hr 30min**
↓
Transfer to **100% EtOH**, cold room, **1hr 30min**
↓
Transfer to **100% EtOH**, cold room, **overnight**

DAY 2

Transfer to 200ml **100% EtOH**, cold room, **1hr**
↓
Transfer to 200ml **75% EtOH+25% Xylene**, cold room, **30mins**
↓
Transfer to 200ml **50% EtOH+50% Xylene**, cold room, **30mins**
↓
Transfer to 200ml **25% EtOH+75% Xylene**, cold room, **30mins**
↓
Transfer to 200ml **100% Xylene**, cold room, **1hr**
↓
Transfer to 100ml **100% Xylene**, cold room, **1hr**
↓
Transfer to 50ml **100% Xylene**, cold room, **1hr**
↓
Transfer to 25ml **100% Xylene**+half jar **paraplast**,
Put the jar on the top of oven (set to 59°C) **overnight**

DAY 3

Pour half wax out and add half new wax in the morning and evening,

Open the cap and keep the jar in 60°C oven **with vacuum on**

DAY 4

Pour half wax out and add half new wax in the morning and evening,
Open the cap and keep the jar in 60°C oven **with vacuum on**

DAY 5

Pour half wax out and add half new wax in the morning and evening,
Open the cap and keep the jar in 60°C oven **with vacuum on**

DAY 6

Pour half wax out and add half new wax in the morning and evening,
Open the cap and keep the jar in 60°C oven **with vacuum on**

DAY 7

Make the tissue block:
Make a small paper boat, then pour some wax and the tissue in the boat
↓
Try to orientate the tissue as desired
↓
Solidify the wax by floating the boat on cool water
↓
Store the block in 4°C

Stock solution:

10x PBS

1.3M NaCl	74g
0.07M Na ₂ HPO ₄	9.94g (If Na ₂ HPO ₄ ·7H ₂ O, then use 18.76g)
0.03M NaH ₂ PO ₄	4.14g (If NaH ₂ PO ₄ ·2H ₂ O, then use 4.68g)

-> Add dH₂O to 1L