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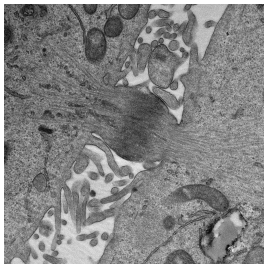
TEM processing for cytoskeleton and matix preservation in tissues V.1



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Abstract

Processing schedule to allow good cytoskeleton and extracelluar matrix preservation and visibility in tissues. Ultrathin sections can be examined in TEM without post-staining.

Image Attribution

Aleksandr Mironov

Materials

Stock HEM buffer (0.2M) in water:

HEPES	170mM
EGTA	20mM
MgCl2	4 mM

25% glutaraldehyde stock solution (EM grade)

16% formaldehyde stock solution (EM grade)

0.2M cacodylate buffer stock solution (pH7.2)

Tannic Acid (Low Molecular Weight) powder

4% osmium tetroxide solution in water

3% potassium ferrocyanide solution in 0.2M cacodylate buffer (pH 7.2).

5% uranyl acetate stock solution in water

100% Ethanol (analytical grade)

100% Acetone (analytical grade)

MilliQ grade water

Epoxy resin kit (valid for TAAB LV or TAAB 812)

Troubleshooting

Safety warnings

Wear protective gear (gloves, labcoat, goggles) when working with toxic materials.

Before start

All tissue pieces have to be cut to small size of about 1-3mm cubed to get enough staining. One of the dimensions should not be more than 1mm, others can be 2-3mm.
Tissue should be dissected as soon as possible after excision from an animal to avoid hypoxia artifacts.

Fixation

1h 15m

1 Fix with 2.5% glutaraldehyde and 4% formaldehyde in 0.1M HEM buffer (pH 7.2) for at least 1 hour. 1h

2 Rinse with 0.1M HEM buffer (pH 7.2) 3×5min. 15m

Post-fixation

4h 45m

3 Incubate with freshly made mixture of 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1M cacodylate buffer (on rotator). 1h

4 Rinse with distilled water 3×5min. 15m

5 Incubate with 1% tannic acid (low molecular weight) in 0.1M cacodylate buffer (pH7.2) for 1h (on rotator). 1h

6 Rinse with distilled water 3×5min. 15m

7 Incubate in aqueous 1% uranyl acetate solution for at least 2hr (better – overnight).* 2h

8 Rinse with distilled water 3×5min. 15m

Dehydration

2h

9 70% ethanol in water (could be left overnight) 20m

10 90% ethanol in water 20m

11 100% ethanol 20m

12 100% ethanol 20m

13 100% acetone 20m

14 100% acetone 20m

Epoxy resin infiltration 1d 1h 30m

15 Prepare fresh resin formulation according to the manufacturer instructions. For better miscibility the resin components (except accelerator) can be warmed up in 60C oven. Mix all components very well by shaking the resin bottle. Leave the bottle with full resin mix in 60C oven for a while (about 10min) to let all bubbles (after shaking) to escape. 30m

16 Incubate tissue pieces in 25% resin mix in acetone 1h

17 Incubate tissue pieces in 75% resin mix in acetone 16h

18 Incubate tissue pieces in 100% resin mix 8h

Polymerization 1d 0h 10m

19 Put tissue pieces into resin- prefilled silicon moulds. 10m

20 Log the mould numbers or put sample ID labels (laser printed on small pieces of paper, font 6pt) inside. Top up moulds with resin so the upper surface would be a bit convex.

21 Put moulds into 60C oven for overnight or longer (can be over a weekend) 1d

22 Ultrathin sections can be examined in TEM without post-staining.