EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes

Protocols:
Bio EM Processing – Start to Finish

This Kirsch Note (KN) provides a general outline of steps taken to chemically prepare samples for TEM and SEM. If there is a number in parentheses at the end of a step, that is a hyperlink to the KN that contains more information on that particular step. Links to the KNs are also listed at the end of the article.

Bio EM Processing

Chemical Processing – TEM Embedment (#2A) – SEM Drying (#2B)

NOTE: Follow all general lab safety protocols. (#1)

1. Samples for TEM, preferably perfused, are placed in a few drops of fixative and cut into small cubes 1 mm^3. If the samples have an orientation, such as skeletal muscle, they should be cut with an aspect ratio such that the narrower edge will form the block face. (#3) (#4)
2. Cut samples should be placed in a suitable vial containing the fixative of choice.
3. The samples are then washed three times in the same 0.1M buffer as used in the fixative.
4. Next the samples are immersed in OsO^4, 2-4% in aqueous or buffer.
5. The samples are wash three more times in DI water.
6. Samples are taken through a graded series of dehydrant, ETOH or acetone, typically starting at 50%, 70%, 80%, 95%, and finally three changes of 100%. **NOTE:** If using ethanol, do not use denatured alcohol. (#5)

NOTE: For SEM, stop here and refer to HMDS (#2B) OR CPD (#6A) (#6B)

7. The samples are now infiltrated with the final embedment resin of choice (see #7A) starting with a 2:1 ratio of final dehydrant to resin followed by a change to 1:1 ratio and finally 2 changes of 100% resin. (#7A) (#7B)
8. Final embedment is done in the same 100% resin as infiltration.
9. Desired embedment mold is selected, a drop or two of resin is put in, the sample placed near the tip, additional resin added to fill, and label is inserted. (#8)
10. Embedment trays are placed in 70° C oven overnight for polymerization.
11. Polymerized blocks are removed for the oven and brought to RT to be removed from mold.

Sectioning – Thick and Thin

1. The blocks are removed from the mold and placed in the ultramicrotome chuck for trimming.
2. A razor blade is used to remove excess resin from the top of the block face until tissue is encountered.
3. The sample should then be faced off with an old diamond or a glass knife to provide a flat reflective surface. (#9)
4. The ROI is identified and excess materials is removed to obtain a rectangle or trapezoid shaped block face ≤ 2 mm^2.
5. The block/chuck assembly is placed in the ultramicrotome arm with the specimen arc adjustment in the vertical position and the degree numbers to the right.
6. A knife is placed in the knife holder and clearance angle set – 4° for glass and 6° for Diatome diamond knives.
7. The knife assembly is advanced manually to a position close to the sample and locked.
8. Observing the block face/knife position the block is rotated to align the bottom edge of the block parallel to the knife edge.

9. The block arc and knife arc are adjusted so a complete section, top to bottom and left to right respectively, will be cut.
10. Using a knife with a boat, add water to the boat and cut sections 0.5 – 2 µm thick.
11. Sections are retrieved from the water, placed on a glass slide, excess water is evaporated and section is stained. (#10A RMC) (#10B Leica)
12. The ROI is selected, and the block is trimmed accordingly if needed. (#9)
13. The block/knife assembly is placed back in the microtome and a thin section knife is inserted into the knife holder, secured, and clearance angle is set.
14. The same process as steps 7-10 are performed, except section thickness is 60-100 nm. (#11)
15. Thin sections are put onto grids for post staining. (#12)

Post Staining

1. Grids are selected to be stained. (#13)
2. A piece of parafilm is taped to a flat surface and a Petri dish cover to cover the film is selected.
3. A drop of fresh UA for each grid is placed on the parafilm and a grid, with section side down, is floated on top of the drop.
4. The Petri dish cover is placed over the drops with grids and a dark cover is placed over the entire area for the time desired for staining.
5. Three beakers are filled with DI water and each grid is dipped 10 or more times in each beaker.
6. Grids are dried and are now ready for lead staining.
7. Another piece of parafilm is taped to the surface and 3-4 pellets of NaOH are placed on the film and covered with Petri dish cover.
8. Fresh filtered drops of lead stain are placed on the film and pre-stained grids are floated section side down on the lead drops for desired duration.
9. Grids are removed and dipped 10 or more times in fresh DI water.
10. Grids are dried and ready to observe in the TEM. (#13)

TEM Observation (#14)

This KN covers general problem solving for TEM observation.

Linked List of KNs Mentioned in Body of Text

#1. General Laboratory Safety
#2A. Biological Processing Steps for TEM
2B. SEM Biological HMDS Processing
#3. Fixatives for Electron Microscopy
#4. Buffers for Electron Microscopy
#5. Dehydrants for Electron Microscopy
#6A. Critical Point Drying with the EMS 3100
6B. Critical Point Drying with EMS Q850
#7A. TEM Embedding Resins
7B. Challenges with Resins
#8. Embedding Samples
#9. Block Trimming for TEM
#10A. Thick Sectioning for TEM using the RMC Power Tome PC
10B. Thick Sectioning using the Leica UC7 Ultramicrotome
#11. How to Thin Section TEM Samples
#12. Three Techniques for Retrieving Thin Sections
#13. Preparation of Post Staining Solutions
#14. TEM Imaging Issues and Solutions

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