Observation of biological specimen by JEM-1400Flash – Flow from sample preparation to observation –

Related product：Transmission Electron Microscope(TEM)

JEM-1400Flash

Introduction

Using an electron microscope allows for observations of micro-structures in a cell which is not possible with an optical microscope. In order to understand the detailed structure of organelles such as mitochondria and chloroplasts, an electron microscope is a powerful tool. However, with TEM, observation of a specimen in a living state is not possible as the observation is performed in vacuum. Also, in order for an electron beam to transmit, specimens need to be thinly sliced.

For this purpose, specimen preparation is a very important step to grasp the structure of the target without artifact. This application note describes the flow of sample preparation for biological specimens, using an example of a plant tissue to introduce data that is obtained by JEM-1400Flash.

Flow of specimen preparation

There are various methods in preparing biological specimens. As examples, we introduce specimen fixation by using a chemical fixation method and ultra-thin sectioning method by using an ultramicrotome. The specimen preparation process follows this order: 1. Specimen cutting, 2. Fixation, 3. Dehydration, 4. Substitution, 5. Embedding, 6. Polymerization, 7. Trimming, 8. Ultra-thin sectioning, 9. Staining. Each process is explained below.

1. Specimen cutting

The specimen is cut into small pieces using a razor blade, so that the chemicals to be used in further processing can easily permeate.

2. Fixation

A process to stop structural change of biological specimens with only a few artifacts. For chemical fixation, fixation is performed twice: prefixation and postfixation. Prefixation is used to fix proteins, and postfixation is used to fix lipids.
Note: Osmium tetroxide is highly volatile and reactive, and can damage the respiratory system, skin, and mucous membranes of the user. So, the process should be performed in a fume food.

Reagents

- Prefixation solution：2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M HEPES*1 (half karnovsky)
- Postfixation solution：1 % osmium tetroxide solution（OsO_4 in 0.1 M HEPES）
- Wash solution：0.1 M HEPES or distilled water
- Sample bottle

*1 HEPES：an abbreviation of buffer solution 2- [4- (2-Hydroxyethyl) -1-piperazinyl] ethanesulfonic acid

3. Dehydration

Use ethanol or acetone to dehydrate water content in the cells. If moisture remains, the remaining water content will prevent polymerization of resin during subsequent resin embedding. Follow the flow below to soak the specimen in each concentration of ethanol to substitute the water content with ethanol.

50 % Ethanol	70 % Ethanol	80 % Ethanol	90 % Ethanol	95 % Ethanol	100 % Ethanol
> 20 min	> 20 min	5 min	5 min	5 min	3 x 5 min

4. Substitution

When dehydrated with ethanol, it is substituted with propylene oxide (PO) or similar as an intermediate agent to blend with the epoxy resin.

Reagents

- PO
- Epoxy resin

※TAAB's EPON 812*2 was used.

1) PO only 2 x 5 min
2) PO：resin＝2：1 2 hours
3) PO：resin＝1：1 2 hours
4) PO：resin＝1：2 2 hours
5) Resin only 18 to 24 hours

－Resin adjustment－

1) Measure EPON 812*2, DDSA*3, MNA*4 in the proportions shown in the table and put them in a beaker.
2) Stir well using a stirrer
3) Add DMP-30*5 and stir
4) Degas the resin and remove air bubbles

Table: Epoxy resin compounding

(%)	soft	medium	hard
EPON812	48	48	48
DDSA	30	19	12
MNA	20	33	40
DMP-30	2	2	2

Reference：TAAB's DATA SHEET 12a

*2 EPON 812：resin,*3 DDSA・*4MNA：hardener,*5DMP-30: accelerating agent

5. Embedding

In order to prepare an ultra-thin section, it is necessary to embed the specimen in hard embedding agent such as resin. Liquid resin will be cured during the polymerization process later.

Tools

- Epoxy resin
- Silicon embedding plate (Fig. 1)

1) Pour the resin
2) Place the specimen at the tip of the silicon embedding plate
3) Let it sit for a while to blend

Fig. 1 Silicon embedding plate

6. Polymerization

Heat the resin to cure, to allow for thin slicing of the resin. Place the silicon embedding plate in an oven and keep it at 60℃ for three days. Stabilizing the temperature during polymerization can prevent polymerization failure of the resin.

Tools

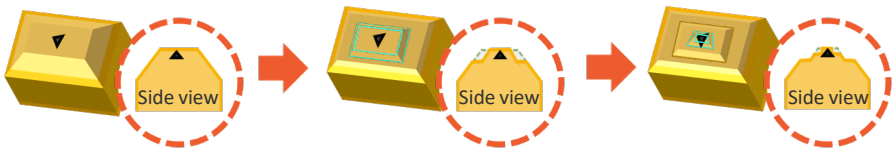
- Oven for resin polymerization

7. Trimming

Trim the specimen under the optical microscope so that the area to be observed has the size to be placed on the specimen grid and shape the surface to be observed.

Tools

- Razor blade
- Optical microscope



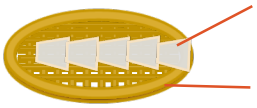
8. Ultra-thin sectioning

Remove the resin to expose the surface of tissue using a glass knife. Then cut into slices thin enough to transmit an electron beam with a diamond knife and an ultramicrotome, and placed on a grid.

Tools

- Ultramicrotome
- Grid
- Diamond knife
- Glass knife
- Eyelash probe (Fig. 2)

Fig. 2 Eyelash probe



9. Staining

This operation is used to enhance the contrast of biological specimens which contain many light elements. The scattering contrast is enhanced by binding heavy elements to the specimen. When staining the ultra-thin sections on a grid, double-staining is performed using uranyl acetate and lead citrate as staining agents. Uranyl acetate stains nucleoplasm and ribosome, while lead citrate stains cell membrane, glycogen granules, and ribosome.

Reagents

- Uranyl acetate *6
- Lead citrate

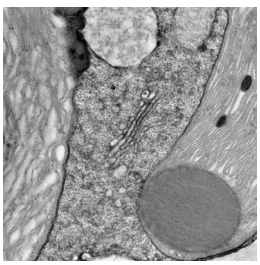
*6 Uranyl acetate is a compound which is an internationally controlled substance that can only be used in licensed facilities. Alternative solutions to uranyl acetate include ytterbium acetate.

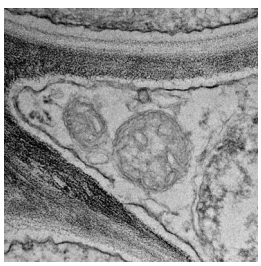
Result

Fig. 3 shows the results of the observation of an ultra-thin section specimen prepared by following the above flow, by using a TEM (JEM-1400Flash).

Fig. 3 TEM images of maple leaf

The membrane structure of the organelle can be clearly captured.
In the left image of Figure 3, the Golgi apparatus can be seen in the center and chloroplasts on both sides.
The image on the right shows the structure of the mitochondrial membrane.





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