Kawamoto's Film Method

Enzyme histochemistry, immunohistochemistry and in situ hybridization are general techniques in biological science. The results depend on the quality of the sections. Therefore, in addition to preserving the fine structure of the tissues, the enzyme activity, antigenicity and tissue components must be preserved. Generally, tissues are treated with a chemical fixative to preserve their structure. Hard tissues are decalcified to permit easy sectioning. The fixation and decalcification usually cause denaturation of proteins, thus resulting in a reduction in enzyme activity and immune reactivity. There is also an accompanying loss and/or dislocation of water-soluble materials from the tissues. The freeze-sectioning technique is one of methods to avoid the denaturation and loss of water-soluble materials. The conventional freeze-sectioning technique is successful in relatively small soft tissues. However there are many types of specimen for which the technique does not work well.

This method was developed in order to produce high quality sections from such troublesome specimens by Dr. Tadafumi Kawamoto (Radioisotope Research Institutes, Tsurumi University, School of Dental Medicine, Yokohama, Japan). This method allows producing very thin fresh sections from hard tissues and large samples such as whole experimental animals. The stained section is permanently preserved with a specially designed mounting medium. The method has the following advantages:

1) Thin complete sections are prepared from large specimens and hard tissues.
2) All the tissue components are almost perfectly preserved.
3) The sections with no distortion are prepared.
4) The sections are used for many kinds of study.
5) This method can be applied to many types of specimens.
6) Immunostaining can be carried out with nonfixed and undecalcified sections.
7) The sections can be permanently preserved with specially designed mounting mediums.
8) The specimens can be examined within 20 minutes.
9) The sections can be used for Laser Microdisection Technique.
10) Neither special training nor techniques are required for this method.
11) The running costs are economical.

This method produces thin frozen sections from whole animals, hard tissues (bone and teeth from rats, rabbits and humans) and plant specimens (leaves, pollen, and grains). By using the following materials (specially designed for this method), one micrometer thick section can be prepared from bones of adult experimental animal (mice and rat) without decalcifying.

1) Embedding medium: SCEM
2) Adhesive film for supporting frozen sections: Cryofilm
3) Disposable tungsten carbide blade: SL series
4) Mounting medium: SCMM

The sections attached to the adhesive film are used for many types of staining such as histology, histochemistry, enzyme histochemistry, immunohistochemistry, and insitu hybridization. The immunohistochemistry can be carried out with nonfixed and undecalcified sections. In addition to these applications, the sections are used for observing the PGF fluorescence. The sections are also usable for studying the distribution of water soluble materials in the tissues. Furthermore, the sections are very useful for gene analysis using LMD technique and for imaging mass spectrometry.

Please see the following book for the details about the latest method or please contact directly to Dr. Kawamoto (info@section-lab.jp).

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