

Hiraoka Staining Kit

Page 1 of 1

A Technique for Multiple Grid Staining and Autoradiography¹

The preparation of ultra-thin biological sections for electron microscopic observation requires that they be mounted on specimen support grids. These grids are very small and fragile so that it is difficult to simultaneously process many of them under similar conditions of staining and fixing. For autoradiography, it is even more difficult as the procedure must be done in a dark room. Various devices have been purported to overcome this difficulty^{2,4} but have not proved to be entirely satisfactory.

The Hiraoka Staining Kit allows simultaneous treatment of up to forty grids while minimizing contamination and mechanical damage of the section.

Each Hiraoka Staining Kit includes five flexible plastic plates each with 40 precision incisions for gripping the grid, a plate holder to facilitate insertion of the grids, and a staining dish which eliminates atmospheric interference.

Grid Supporting Plate and Plate Number

The plate, which is of flexible plastic construction, supports a large number of grids in a small surface area. Precision cut slots are made in the plate to allow firm gripping of the grids. Alphabetical and numerical indexing on the face of the plate allows easy identification of each grid. In order to insert the grids in the supporting plate, the incisions must be slightly open. This is done by flexing the plate in a regular convex position and then snapping it into position in the plate holder. The grids can then be positioned by eye or with a low power binocular microscope. To remove the plate, gently slip it out of the holder from either end. The plate then resumes its flat shape and firmly grips the grids so that they will not fall out when inverted or washed under a stream of water.

Staining Dish

To facilitate staining, a small volume plastic dish is provided. It is manufactured from solid polyethylene. The supporting plate is inverted over the staining dish for staining sections so that there is no opening between the plate and the brim of the dish. The dish is filled with staining solution and the flat plate holding the grids is gently lowered and positioned on the top of the dish. This avoids contact with air and CO₂.

Carbon Coating

A thin layer of carbon can be applied to grids without removing of the grids from the supporting plate. Setting the carbon

source evaporator 15 cm distance and at a 50° to 70° angle to the plate allows the multiple coating of grids.

Application of the Emulsion for Autoradiography

Holding the supporting plate vertically with the sections facing upwards, a fine pipette is used to place a small drop of diluted, 40°C - 45°C emulsion on each grid. Then the plate is placed in a horizontal position with the grids vertical and the excess emulsion is quickly removed by touching the lower ends of the grid with a strip of filter paper. The plate is returned to a vertical position and allowed to dry to room temperature. Glauert⁵ has suggested techniques for handling the photographic emulsion.

In contrast with previous methods, the Hiraoka technique greatly improves the preparative procedures involved in routine staining of grids. The one-time handling of the grids for liquid treatment procedures greatly decreases the possibility of mechanical damage and contamination of the sections.

References

1. Hiraoka, J., *Stain Tech.*, **47**, 297 (1972).
2. Aita, S., *J. Electron Microscopy*, **18**, 137 (1969).
3. Hay, E.D. and Revel, J.P., *J. Cell Biol.*, **16**, 29 (1963).
4. Salpeter, M.M. and Bachmann, L., *J. Cell Biol.*, **22**, 469 (1964).
5. Glauert, A.M., *Techniques for Electron Microscopy*, 2nd ed., Blackwell Scientific Publications, Oxford, England, 1965, p. 282.

Ordering Information:

Cat. #	Description	Size
04635	Hiraoka Staining Kit	1 kit

Replacement items for the Hiraoka Kit

Cat. #	Description
07344	Hiraoka staining dish
07346	Hiraoka supporting plate
07345	Hiraoka plate holder

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
In Germany FAX: (49) 6221-764620