

## Tri-Acetate Replicating Sheets

(20 Sheets, 130 $\mu$ m x 150mm x 150mm)  
Product Number 44848-4

### Electron Microscopic Specimen Preparation of Tri-Acetate Replicas

#### Application:

The Tri-Acetate technique is a method for making electron microscopic specimens, which corresponds to a great extent to the indirect replica method described by Mahl and Konig. Tri-Acetate is used instead of varnish composed of pyroxylin and amyl acetate for taking replicas. The preparation time is thereby greatly reduced while the quality of the substances remains the same. Whereas pyroxylin-amyl acetate (zaponlac) replicas may not be removed from the base until they have dried for 10 hours, the fast drying Tri-Acetate foils may be removed after only 3 to 5 minutes, without having to expect deformations. The entire preparation process from taking the replica to finished specimen requires approximately two hours.

#### Protocol:

- For reproducing smooth and moderately rough surfaces, foil of 40 $\mu$ m thickness is suitable while 130 $\mu$ m Tri-Acetate foil should be used for rougher surfaces. Depending on the size of the surface to be replicated cut small foil pieces and clean carefully with a soft brush removing any adhering particles. Wet the preparation spot, which is to be replicated, with a few drops of acetone P.A. (spray from a fine glass capillary tube). If too much solvent is used, there is the danger that the thin foil expands too much and then tears when it is removed. Before the solvent can evaporate apply the Tri-Acetate foil to the specimen surface with as little pressure as possible and wet the foil with acetone. Always use the dull side of the foil for taking replicas. The dried Tri-Acetate replica can be removed from the base after 3 to 5 minutes.
- Coat the foil with a thin metal or metal oxide layer in an evaporation unit. A line of evaporators can be found on our web site or in our catalog. After oblique shadowing (angle 15 to 25° for smooth objects, 30 to 45° for rough objects) and application of a very thin vertical vaporization layer, coat the foil on the vaporized side with a paraffin film of approximately 0.3 mm thickness in order to render the thin vaporization layer more solid. This layer can easily tear during the subsequent solution process. When the molten paraffin (melting point approximately 52 to 53°C) is applied from an open glass tube, make sure that no paraffin is applied to the back of the foil.

44848-4 TN V1 09142010

Page 1 of 2

**Note:** For the dissolving processes described below, make sure that there is absolute freedom from vibrations. The slightest oscillation causes tearing of the thin replica foils and renders the replica useless due to the formation of folds.

- Place the matrix cautiously into a methylacetate bath saturated with paraffin (paraffin side upwards) until the violet color of the Tri-Acetate foil has disappeared.
- Remove the matrices after 20 minutes with a glass siphon and rinse again for 5 to 10 minutes in a second fresh methylacetate bath.
- Then, dry matrices in a dust free place on filter paper, with the shadowing layer upwards and the paraffin layer down-wards.
- Then, cut specimens with a razor blade into small squares having an edge length of approximately 2 mm, place these (vaporization layer downwards) on the surface of clean electron microscopic specimen carriers (specimen diaphragms or grids), and weight them by placing a small piece of brass wire mesh (0.3 mm wire, 0.6 mm mesh width, size 2 mm x 2 mm) on them.
- Place the specimen diaphragms, which have been weighted in this manner, in a bath with heated toluene p. A. with forceps for removing the paraffin. Leave them in the toluene bath until the paraffin has been removed completely (approximately 10 minutes). Remove all paraffin traces in a second toluene bath.
- Take out specimen carrier cautiously and dry carefully. The replica foil may easily tear if the liquid, which is contained in that diaphragm cone, is sucked out suddenly. Therefore, place the diaphragms on the filter paper first with one edge only. When the specimens are dry, the wire meshes can be thrown off easily.
- Contaminated or damaged specimens can usually be eliminated by light microscopic control.