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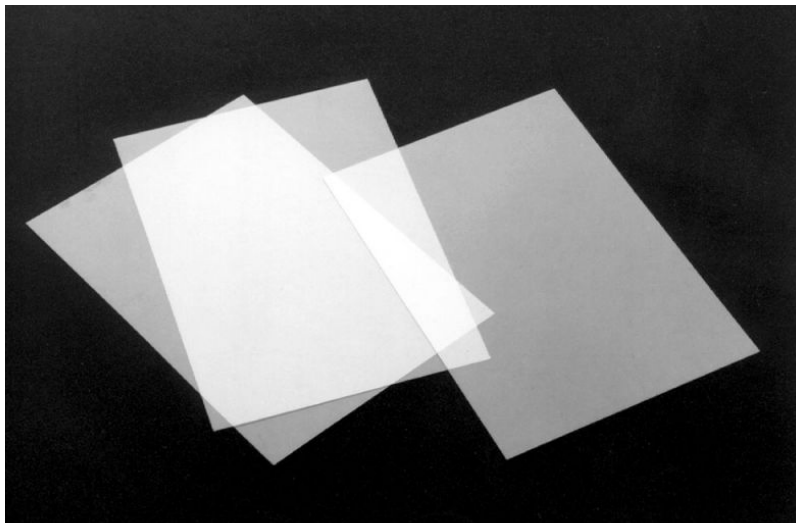
Melinex Film

AGL4103



Melinex is a polyester film. Thickness 175µm. The procedure is as follows:

1. Cut out coverslips of the required size from the Melinex sheet (e.g. 20mm x 20mm). It is important to do this before growing the cells.

2. Wash with ethanol, by wiping with tissue soaked in ethanol.

3. Sterilise in ethanol (70%), 2 or more changes, or keep in 70% ethanol.

4. Air dry and keep sterile.

5. Incubate in serum-containing medium in a Petri dish overnight at 37°C (this removes any toxic substances).

6. Remove medium, and wash in fresh medium.

7. Add cells and medium and incubate in the usual way.

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Fixation for Electron Microscopy

1. At the end of the culture period, add sufficient 25% glutaraldehyde to the medium in the culture dish to obtain a final concentration of 2.5% glutaraldehyde. Alternatively, the medium is gently removed (so as not to disturb the monolayer of cells and is replaced with an equal quantity of warm glutaraldehyde fixative (2.5% glutaraldehyde in 0.09M cacodylate buffer, pH 7.2, containing 3mM calcium chloride).

   Important: Fixation must be done at the incubation temperature; warm the fixative in the incubator before use and add it to the culture in the incubator (it is helpful to have a small incubator in the fume hood).

2. Allow to fix for 30 minutes. Remove the dish from the incubator and replace with buffer (0.1M cacodylate buffer, pH 7.2, containing 3mM calcium chloride), at room temperature. Remove the fixative as gently as possible.

3. The fixed cultures can be stored in buffer at 4°C; ensure they do not dry out.

Subsequently, the cells on the Melinex coverslips are post-fixed in osmium tetroxide, washed in water, stained with uranyl acetate, dehydrated in ethanol, and embedded in Araldite. For the final embedding the coverslip is inverted over an Araldite-filled plastic container (e.g. the cap of a specimen tube). After the resin has polymerised, the Melinex is easily peeled away from the Araldite.

Another layer of embedding medium can be added to form a ‘sandwich’ with the cell monolayer in the centre. This is carried out in a flat embedding mould made out of Al foil.

Melinex does not dissolve in propylene oxide.