Polysciences, Inc.

Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1-800-523-2575
FAX 1-800-343-3291
Email: info@ polysciences.com
www.polysciences.com

Europe - Germany
Polysciences Europe GmbH
Handelsstr. 3
D-69214 Eppelheim, Germany
(49) 6221-765767
FAX (49) 6221-764620
Email: info@ polysciences.de

Diethylene Glycol Distearate

A Removable Embedding Medium

Background:

Diethylene Glycol Distearate (DGD) can serve as a removable embedding medium for both thin and thick sections (i.e. > 1 micrometer). After removal of the embedding medium, the thicker sections provide proportionally a greater amount of spatial cues for the organization of components within the section. For example, in thicker sections, the exterior surface of mitochondria can be observed, whereas in a resin-embedded thin section, the mitochondria are most frequently observed in cross section. Cells that contain large amounts of acellular materials such as yolk, which shrinks during processing, may cause a breakage site between the cytoplasm and each body of yolk. DGD medium has been used successfully for the ultrastructural analysis of various somatic cells grown in culture[1,3] and on the eggs and embryos of mammals.[2,5] Additionally, this technique has been used successfully to prepare tissue for immunofluorescent localization of cytoskeletal components[6] and to prepare tissue for *in situ* hybridization with nucleic acid probes.[4]

Instructions for Use:

After fixation, dehydration is achieved through a graded series of alcohol. A minimum incubation time of two hours with several changes of 100% ethanol should be conducted before going into the transition fluid. The transition fluid is n-butanol and a graded series should be used to transfer the specimen from 100% ethanol (at least a 1:1 mixture of n-butanol to 100% ethanol). Subsequently the specimen is transferred into 100% n-butanol with several changes of 100% n-butanol over a minimum of two to three hours. Processing should be done either in Nalgene plastic or in glass. During the final change of n-butanol the sample should be placed in an oven adjusted to 70°C for about 15 minutes to warm the tube and the n-butanol prior to infiltration into the embedding medium.

Embedding:

DGD is a white waxy solid which should be fully melted at 70°C prior to use. Addition of 0.3% DMSO to the DGD prior to use reduces the shrinkage which occurs as the block solidifies. Occasionally, a white flocculent will appear in the molten DGD, particularly if left in the molten state for several days. This can be removed by filtration through a Whatman #1 filter conducted in the oven.

A 1:1 premixed solution of n-butanol to molten DGD is added to the samples and allowed to incubate for at least 45 minutes. Subsequently, this 1:1 mixture is replaced with several changes of DGD (containing 0.3% DMSO) and allowed to incubate for a minimum time of two hours (dependent upon the size of the specimen). Infiltration in a vacuum oven can be used to promote penetration of the DGD if it is needed.

Infiltration in flat embedding molds is recommended because the solidified DGD is very brittle and removal from embedding mold such as BEEM* capsule may cause the block to fracture or crumble during sectioning. Flat embedding molds with 4mm depth provide enough DGD to compensate for any shrinkage and are shallow enough that the block can be removed from the embedding mold without breakage. To orient the specimen within the embedding mold prior to beginning the solidification process, place heat lamps around a dissecting microscope to heat the stage up to 70°C. As the block cools and contracts, a little molten DGD should be added to the edge of the mold opposite the specimen so that when the block is completely solidified it has a fairly flat upper surface. Do not place the mold on ice or in the refrigerator to promote rapid solidification as the process usually causes block fractures. Instead, the block should be left to cool at room temperature. To remove the block from the embedding mold, the edges of the mold surrounding the block should be flexed slightly to cause the adhesion points between the block and the mold to break. After the block has been thoroughly loosened, it should be lifted out by bending the mold. Do not stress the block, otherwise, it will crack. Note that once the DGD had solidified, re-melting will damage the specimen for ultrastructural analysis.

Sectioning:

It is recommended that the block be placed in a vise-type chuck for microtome. When trimming, make shallow cuts, since a deep cut can cause enough stress to fracture the block. It is preferable to start with specimens that are small enough such that a layer of DGD can be left around the specimen. The DGD surrounding the specimen during sectioning also helps promote

the formation of ribbons and appears to enhance the integrity of the specimen as it floats on the water. The hardness of block depends on the room temperature, preferably 75°C or cooler. Avoid touching the block with fingers as this softens the block. Once trimmed, sections can be obtained as with any standard resin block, except that the knife angle should be set at 10°. Sections floated on water will provide interference colors to give an indication of the section thickness and the relative thickness of one section to another. The sequence of interference runs from silver, to gold, to green, to blue, and then dark gold, green, and blue and this sequence will repeat itself for an additional cycle. The integrity of silver sections is rarely adequate, however, it is possible to obtain acceptable gold sections. Since this embedding medium will be removed it is also possible to use sections that are much thicker than this. For example, the subsequent blue or green or even the dark gold sections can provide excellent images of the tissue of interest. In detergent-extracted specimens[1], sections that are over one half a micron thick can still be easily penetrated by an electron beam of 80 kilovolts. The sections float on a trough filled with water and these ribbons can be picked up on grids that are formvar-coated, carbon-stabilized and treated with 0.1% polylysine. It is recommended that the polylysine be applied immediately before use. A problem that is often encountered is that during the removal of the embedding medium from the sections, some of the sections float off of the grid even though the grids are polylysine coated. To improve adhesion of the sections to the grids it is recommended that after their initial collection they be dried completely and then a droplet of polylysine added to the grid and allowed to incubate for several additional minutes. The excess is then removed and specimen dried again overnight in a vacuum desiccant.

Removal of the Embedding Medium From Sections:

After thoroughly drying the grids, the DGD is removed from the sections by immersing for one hour in n-butanol as gently as possible so as not to dislodge the sections from the grid. The grid can be agitated one time by gently swirling the petri plate containing the n-butanol and then allowed to sit for an additional 15 minutes. Transfer the grids to a 1:1 mixture of 100% n-butanol to 100% ethanol for an incubation time of 15 minutes. Subsequently, the grids are transferred to a solution of 100% ethanol and allowed to sit for an additional hour, swirled gently, and then allowed to sit for an additional 15 minutes. At this time, the grids can be placed in a holder for critical point drying and subsequently dried through the CO_2 critical point.

TEM Examination:

Grids should be retained in a vacuum desiccator until use. During observation it is recommended that the emission current of the microscope be reduced to one half of what would be typically used for a resin-embedded section and that the cold trap be completely filled. This appears to promote stability of the specimen in the beam. If the specimen remains very unstable, a thin layer of carbon can be evaporated on to the surface of the specimen prior to observation. Embedment-free sections have particularly high contrast and we have found it necessary to adjust the developing conditions to provide an image which is printable. For this purpose we recommend Kodak EM Film emulsion number 4489 be developed in a freshly made solution of D-19 diluted 1:8 with water and developed for a total of 8 minutes. Washing and fixation follow standard procedures.

Staining:

Staining is not necessary because these specimens have intrinsically high contrast since there is no embedding resin present and the image is created by the scattering of electrons by the biological material in a vacuum. Post fixation with osmium tetroxide can be omitted but osmium fixation promotes stability under the electron beam and is recommended.

Prepared for Polysciences by David G. Capco

References:

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3. He, D., et al., (1990). J. Cell Biol. 110-569.
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5. McGaughey, R.W. and D.G. Capco. (1989). Cell Motil., Cytoskel. 13:104.
6. Valdimarsson, G. and E. Huebner (1989). Biochem. Cell Biol. 67:242.

*"BEEM" is a registered Trademark of Better Equipment for Electron Microscopy, Inc.

Ordering Information:

Cat. #	Description	Size
01873	Diethylene Glycol Distearate	500g
00202	n-Butanol	1kg
09730	Poly(lysine hydrobromide) 0.1%, MW 60,000-120,000	25ml 250ml
02615	Flat Embedding mold	1ea

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