U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Dunhua N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

TECHNICAL DATA SHEET 279

PEG 4000, EM Grade

BACKGROUND

Polyethylene glycols (PEG) are designated by a number that roughly represents the average molecular weight. PEG 4000 has a melting point of 53° to 56°C and is easily extracted by common solvents. It permits the viewing of resinless sections, thus alleviating the need for heavy metal staining. Because there is no resin to scatter electrons, and no contamination from staining, the cytoplasmic ground substance and cellular organelles are seen with greater clarity than in comparable EPON sections.[1-5] This procedure is useful also for immunocytochemical and X-ray microanalytical techniques.

Resinless sections obtained by the PEG method are also an alternative to ultracryomicrotomy for exposing cell contents which permits direct reaction with antibodies.[6]

To embed in PEG 4000, tissue is fixed and processed by established TEM procedures up to the final 100% ethyl alcohol step in dehydration.

According to the method of Wolosewick,[4] the tissue is transferred to a 1:1 mixture (vol/vol) of 100% ethyl alcohol and PEG 4000 for an initial infiltration of half an hour. The tissue is then transferred to fresh vials containing 100% melted PEG 4000 for one hour. This step is repeated, total infiltration time should be 2 1/2 hours. While infiltration is proceeding, gelatin capsules are put into the same 60°C oven to dry and warm. The capsules are filled with melted PEG 4000 and, following the infiltration period, the pieces of tissue are transferred to the capsules and allowed to sink to the bottom. Capsules are then removed individually from the oven, lowered and solidified in stirred liquid nitrogen for 15 seconds or put into a -20°C freezer for 15 minutes. Complete dehydration and infiltration through 100% PEG 4000 is necessary to produce solidified blocks hard enough for thin sections. Blocks are then ready to be sectioned. The block is trimmed in the same manner as an EPON block. Sectioning has been most successfully done on most microtomes.

TWO METHODS AVAILABLE FOR PICKING UP AND MOUNTING PEG SECTIONS:

1) Sections are cut on a dry knife and picked up with a hair and flattened out on a formvar-carbon coated grid. To assure section attachment, grids are put into a 60°C oven for 7 minutes. The grids are then placed in a critical point dryer grid holder, submerged in 95% ethanol for 15 minutes to dissolve out the PEG 4000. The 95% ethanol is gradually replaced with 100% ethyl alcohol and then dried in critical point apparatus. Drying may also be done in a desiccator which is being pumped to vacuum during the entire drying process. However, the critical point drying is optimal. Sections are ready for viewing. Grids are best kept in a desiccator until ready for viewing.

2) Sections, freed from the knife edge with a hair, can be picked up with a drop of 40% sucrose or 40% PEG 4000 on a 3 mm loop. The sections can be viewed through the drop of sucrose or PEG as an approach is made to pick up the sections from the knife edge. The sections will be drawn up into the drop when the drop is but a few millimeters above the sections. The drop with the sections is transferred by touching the loop to a polylysine-treated (0.1% polylysine, adjusted to pH 8.5), formvar-coated grid. The sections will adhere to the grid. The grid is then immersed in buffer or H_20 and kept submerged until all sectioning has been completed. The sucrose and PEG are washed out of the sections by submerging the grids in warm (57°C) water or buffer for about 15 minutes. Proceed with critical point drying as in #1.

Sections to be used for immunoctyochemistry are mounted as described in Methods #1 and #2 using Poly-L-lysine coated formvar-carbon gold or nickel grids or coverslips for TEM and LM, respectively. Sections mounted according to Method #1 are rehydrated, treated as necessary, dehydrated again and critical-dried. Using Method #2, immunocytochemistry can proceed directly from either H_20 or buffer. Dehydration and critical point drying complete the procedure.

Recently, PEG 4000 sections have been used in X-ray microanalysis. For sections mounted through Method #1, formvar-carbon beryllium slot grids are used instead of copper grids and are placed in a chemically inert multiple grid holder for critical point drying.[7]

PEG embedding is also useful as a method for sample correlation of LM, SEM and TEM images.[8]

Polysciences, Inc.
Chemistry beyond the ordinary

U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Dunhua N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

TECHNICAL DATA SHEET 279

HANDLING PRECAUTIONS:

The full chemical, physical and toxicological properties of PEG 4000, E.M. Grade, are not known. Avoid contact with eyes or skin or respiratory tract. Wear protective goggles and gloves. Avoid breathing vapors and mist. Use only with adequate ventilation. In case of accident, immediately flush either eyes or skin with plenty of water for at least 15 minutes; for eyes, get medical attention. Remove contaminated clothing and shoes at once and wash them thoroughly before re-use. Wash immediately after handling.

ORDERING INFORMATION:

Cat. #	Description	Size
16861	Poly(ethylene glycol), Waxy Solid, MW 4000 (PEG 4000)	250g
09730	Poly(lysine hydrobromide), 0.1% aqueous	25 cc
04672	Polyvinyl formal solution (Formvar Solution), 0.5% by wt. in ethylene dichloride	100g
00403	Dental Wax	1lb 5lb

REFERENCES:

1. Becker, R.P., et al., 39th Ann. Proc. E.M. Soc. Amer., Atlanta, GA (1981).
2. Kondo, H., et al., J. Neurosci., 2, 57 (1982).
3. Wolosewick, J.J., J. Cell Biol., 86, 675-681 (1980).
4. Ibid., 95, 467a (1982).
5. Wolosewick, J.J., et al., Biol. Cell., 44, 85 (1982).
6. Kondo, H., J. Elec. Microsc. Tech., 1, 227-241 (1984).
7. Becker, R.P., et al., J. Cell Biol., 95, 101a (1982).
8. Kondo, H., J. Elec. Microsc. Tech., 2, 457-462 (1985).

Acknowledgement: Virginia Kriho, Department of Anatomy, University of Illinois