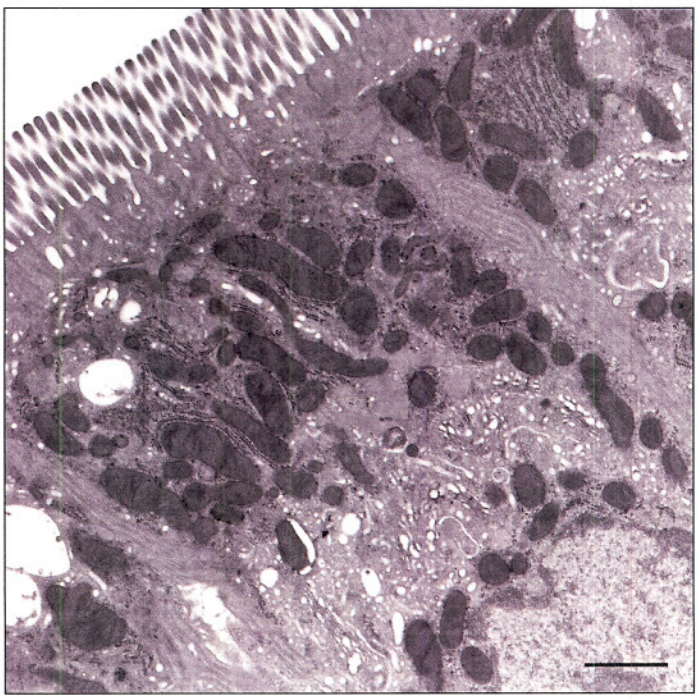
LR Gold Embedding Resin, 500ml
Product No 18183



Intestinal epithelial cell from rat, embedded in LR Gold resin.
Original mag. x 21,400. Bar = 1µm. Picture courtesy of Mark Berryman.*

TISSUE

Tissue samples up to 5 x 5 x 5 mm have been successfully processed using the following schedule. However, it is recommended that tissue specimens of maximum 3 x 3 x 3 mm are used. As a general rule the smaller the specimen, the more efficient the impregnation. The thickness of the tissue is particularly important when polymerizing darkly colored tissue such as liver and spleen, because complete polymerization depends on the blue light from the light source being able to penetrate the full thickness of the tissue. Tissue used is fresh and unfixed.

PROCESSING

Processing is performed in 10 ml vials with tight-fitting lids on a rotary agitator. The fluids involved are maintained in bulk at the sub-zero centigrade temperatures required. Also, the final resins, it must be remembered, are sensitive to prolonged light exposure and are therefore stored in the dark and handled as infrequently as possible. We recommend the use of polyvinyl pyrrolidone (PVP) to protect unfixed tissue from osmotic changes during processing. We have used PVP with an approximate molecular weight of 44,000. This can be dissolved in methanol, water and the London Resin Gold monomer. Concentrations of 50% w/v are possible in the methanol mixtures; however, at low temperatures the resulting viscosity is impractical. The following schedule shows the PVP concentrations recommended, with the resulting LM

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work being very satisfactory. It must be said however, that the addition of PVP in different concentrations may further improve morphology especially in the EM.

FRESH TISSUE†

50% methanol + 20% PVP	0°C	15 min
70% methanol + 20% PVP	-25°C	45 min
90% methanol + 20% PVP	-25°C	45 min
50% LR Gold monomer/50% methanol + 10% PVP	-25°C	30 min
70% LR Gold monomer/30% methanol + 10% PVP	-25°C	60 min
100% LR Gold monomer	-25°C	60 min
100% LR Gold monomer + initiator	-25°C	60 min
100% LR Gold monomer + initiator	-25°C	overnight
100% LR Gold monomer + initiator	-25°C	20-25 hour polymerization

†If fixed tissue is processed omit the PVP

POLYMERISATION

The addition of a light sensitive initiator is needed in order to polymerize the resin and we recommend BENZIL, an alphadiketone, at a concentration of 0.1% w/v. The principle is shown in the diagram below.

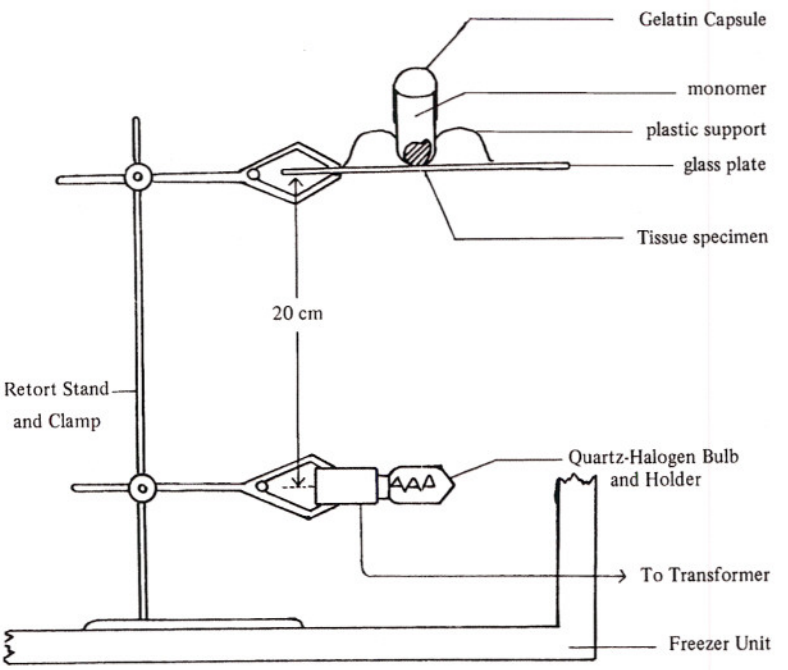

PHOTO CRYOPOLYMERIZATION UNIT

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The gelatin capsules are 00 size and the plastic support is a modified heamagglutination tray. The bulb involved here is a Thorn projector lamp (A1/209 FDX, 12V 100W). We have found that 7 to 9 V will cause solidification within 24 hours. Like many acrylic resins oxygen will inhibit polymerization, therefore the capsules are filled completely and lids fitted. Paper labels can be inserted into the capsules. 9 capsules (3 x 3) may be polymerized at any one time. If the upper surface is still soft after 24 hours this can be trimmed off or hardened in daylight for a few hours prior to peeling off the gelatin. The blocks once polymerized need not be stored cold, however, it may prolong the activity of some enzymes to do so.

The enzyme histochemistry performed to date using the resin has involved conventional reagents, times of reaction and temperatures (see Thompson and Germain, Histochemical Journal Vol. 15, No. 12, December 1983.)

For room temperature polymerization, using a peroxide/amine cure, add to pure LR Gold either 1% of dry benzoyl peroxide or, more safely, 1.5% of benzoyl peroxide paste (60% in dibutyl phthalate). Infiltrate with pure LR Gold solution adding only the peroxide mix prior to polymerization. To reduce curing exotherm, cool mold in ice water. To accelerate cure, add 1 drop of LR White accelerator to 20ml LR Gold resin/benzoyl peroxide mixture. To U.V. light cure LR Gold, add Product No. 18186, benzoin methyl ether. The precise concentration will depend on the power and emission spectrum of your UV lamp. However, a useful starting concentration would be 0.5%. Up to 66 capsules can be cured at a time in the PELCO UVC2 Cryo Chamber.

PELCO UVC2 Exterior/Control Panel

PELCO UVC2 Interior

PELCO UVC2 Cryo Chamber, Product No 6202 and 6202-220

The cross link density of the final resin is important. If stains are not penetrating sufficiently quickly, reduce the benzil concentration rather than the light intensity or exposure time.

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SECTIONING AND MOUNTING

After polymerization the LR Gold blocks can be stored, handled and cut at room temperature. Cutting should preferably be done using a motorized microtome and glass knife. Sections may be cut dry, picked up and placed free-floating into incubating medium or buffer wash for enzyme histochemistry or immunocytochemistry.

It is not advisable to mount sections onto slides before reacting, since this involves heat and would be deleterious to the unfixed proteins. The section can of course be mounted in the usual way after enzyme histochemistry or immunocytochemistry has been carried out.

ORDERING INFORMATION

Product No.	Description
18183	LR Gold Resin, 500ml
18186	Benzoin Methyl Ether, 10g UV Catalyst for LR Gold Resin
81990 to 95	Barrier Gloves, Sizes 6 to 11
81860, 1, 2, 3	Neoprene Gloves, heavy duty; small, medium, large, X-large
81840, 1, 2, 3	Latex Gloves, powder-free; small, medium, large, X-large
6202	PELCO UVC2 Cryo Chamber, 115VAC, 60Hz
6202-220	PELCO UVC2 Cryo Chamber, 220VAC, 50Hz

CAUTION All methacrylates (acrylics) should be considered hazardous. Direct contact and inhalation should be strongly avoided. While moderately toxic and allergenic, high concentrations may be very harmful to tissue. In addition, the methacrylates are combustible and vapors may be explosive.

All acrylics should be stored in completely airtight vessels. Experiments involving the methacrylates should be conducted only under chemical fume hoods and the user should wear the appropriate protective clothing including gloves, barrier creams, safety goggles, and film forming wound sprays where necessary. Order of preference of glove material appears to be Butyl over neoprene and latex, and these over Nitrile or vinyl gloves. In addition 4H gloves from Safety 4A/S in Lyngby, Denmark provide apparently superior protection over all of the standard glove materials. Latex gloves are preferred over vinyl since they fit more snugly around the fingers and are thicker, making them less vulnerable to mechanical damage. Barrier gloves used inside nitrile gloves withstand methyl methacrylate for up to 8 hours.

Waste materials containing methacrylates should be stored in an airtight container in the refrigerator. Every effort should be taken to assure that the storage cabinet, including refrigerators, are sparkproof so as to avoid accidental ignition of stray acrylic vapors. In the event of direct contact with the skin, the affected area should be immediately wiped dry with clean, dry paper towels followed by a thorough washing with soap and water. Never use an organic solvent to clean embedding media components from the skin.

Tobler M and Freiburghaus AV, 1990. Occupational Risks of (Meth) acrylate Compounds in Embedding Media for Electron Microscopy. J Microscopy 160(3): 291-298.

*Berryman MA, Porter WR, Rodewald RD, Hubbard AL, 1992. Effects of tannic acid on antigenicity and membrane contrast in ultrastructural immunocytochemistry. J Histochem Cytochem 40(6): 845-857.

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