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Lowicryl, Low Temperature Embedding Resins

Lowicryl KM4, HM20, K11M and HM23 are highly cross-linked acrylate and methacrylate based embedding media which have been designed for use over a wide range of embedding conditions.[1-3] These resins have been formulated to provide low viscosity at low temperatures: K4M is usable down to -34°C, and HM20 to -70°C. K11M and HM23 have been developed with freezing points that allow applications down to about -60°C and -80°C, respectively. All Lowicryl media exhibit low viscosity at temperatures as low as -35°C. The investigator also has a choice of either polar (hydrophilic; K4M and K11M) or non-polar (hydrophobic; HM20 and HM23) embedding media.

Lowicryl resins are photopolymerized by long wavelength (360nm) ultraviolet light. Since the initiation of the photopolymerization is largely independent of temperature, blocks may also be polymerized at the same temperatures which are used for infiltration. A rapid polymerization method has also been reported.[4]

Proteins and lipoproteins are denatured in organic, water-miscible fluids which are involved in conventional embedding techniques.

The hydrophilic properties of K4M and K11M provide two distinct advantages. During dehydration and infiltration, the specimens may be kept in a partially hydrated state since K4M and K11M may be polymerized with up to 5% (by weight) water in the block.

Secondly, K4M and K11M are particularly useful for immunolabelling of sections using specific antisera, lectins, and colloidal gold particles.[5-8] The use of K4M or K11M results in better structural preservation,[9] improved preservation of antigenicity and significantly lower background labelling.[10-15]

HM20 or HM23 can be used to produce high contrast images of completely unstained thin sections in the Scanning Transmission Electron Microscope by Z-contrast.[16] HM20 and HM23 are particularly suitable for dark-field observation because of their low density, as compared to conventional embedding media. They can also be used routinely at temperatures as low as -70°C. At these low temperatures, biological material is stabilized and may even retain its bound water conditions. All Lowicryl resins can be used for freeze-substituted samples.[17]

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Preparation of Lowicryl Media

The Lowicryls are provided in a highly purified 3-component system: a) crosslinker, b) monomer mixture, and c) initiator. The resin is stabilized, but it is not necessary to remove the stabilizer before use. By varying the ratio of monomer to crosslinker, one can easily tailor the resin hardness to the needs of a particular specimen. (More crosslinker produces harder blocks.)

For example:

- For HM20, the crosslinker concentration may be varied from 5 to 17 weight% (1.0 to 3.5g/20g resin).
- For K4M, the crosslinker concentration may be varied from 4 to 18 weight% (0.8 to 3.6g/20g resin).

The resin should be prepared in a brown glass container or otherwise protected from direct light. All the components are miscible with each other. Avoid excessive stirring which may result in the incorporation of oxygen into the resin, thereby interfering with the polymerization. Gentle stirring, with a glass rod for 3 to 5 minutes, or mixing with a stream of dry nitrogen gas bubbled through resin, is recommended.

The mixtures below give blocks with medium hardness for Ultraviolet Polymerisation:

K4M		HM20		K11M		HM23**	
Crosslinker A	2.70g	Crosslinker D	2.89g	Crosslinker	1.0g	Crosslinker	1.1g
Monomer B	17.30g	Momomer E	17.02g	Monomer	19.0g	Monomer	18.9g
Initiator C*	0.10g	Initiator C*	0.10g	Initiator	0.1g	Initiator	0.1g

*For polymerization of K4M or HM20 between -50°C to 0°C. Above 0°C, the initiator can be replaced by the same amount of benzoin ethyl ether. Room temperature polymerized blocks can be ready for sectioning after a few hours.

**Above -50°C Benzoin monomethyl-ether and below -50°C Igracure 651.

Any of the standard aldehyde fixation procedures may be used. Since the resins are in most cases photopolymerized, the use of fixative which also have staining properties (e.g. osmium tetroxide) is not generally recommended.[18]

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A representative dehydration infiltration schedule for ethanol, which may be used for Lowicryl resin, is given as follows:

Ethanol, Vol%	Temperature	Time
30%	0	30 min.
50%	-20	60 min.
70%	-35 (-50)*	60 min.
95%	-35 (-50 to -80)*	60 min.
100%	-35 (-50 to -80)*	60 min.
100%	-35 (-50 to -80)*	60 min.

Resin: Ethanol, vol: vol	Temperature	Time
1.1	-35 (-50 to -80)*	60 min.
2.1	-35 (-50 to -80)*	60 min.
Pure resin	-35 (-50 to -80)*	60 min.
Pure resin	-35 (-50 to -80)*	Overnight or 4-16 hours.

*Suggested steps for work at a lower temperature – possible only with HM20 (-70°C), HM23 (-80°C) and K11M (-60°C).

At all temperatures below 0°C, care must be taken not to allow the residual water in the specimen to freeze during the dehydration step. A variety of schemes can be developed for any other temperature or polar dehydrating agent, as long as solubility allows it, including freeze substitution methods.

The biological consequences of using even lower temperatures need to be explored. Nothing precise is known, as yet, about the completeness of dehydration at these low temperatures. Lack of success, due to a loss of water, e.g. in the pyramid of the block, should not automatically be interpreted as “bad infiltration”. The demonstrated persistence of lipids at lower temperatures seems not to cause problems of infiltration.[19]

A rapid method of fixation and embedding that avoids low temperatures using HM20 is reported.[20]

Polymerization:

Resins are polymerized by indirect long-wave length UV-irradiation 360nm 2x15 watt (Philips TLAD 15W/05 or equivalent) at -30°C to 40°C at a distance of 30-40cm for 24 hours. Hardening can be done in filled to capacity gelatin or capsules. Slow polymerization, particularly using diffuse radiation, produces superior blocks without severe shrinkage effects. Sectionable preparations can be produced in as little as 12 hours, though the sectioning quality improves when they are further hardened under UV light at room temperature for 2-3 days.

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Sectioning and Staining:

Lowicryl easily yields silver to grey sections on diamond or glass knives. Optimal staining can be done easily with uranyl acetate and lead citrate. Oxidising heavy metal stains like OsO_4 or $KMnO_4$ produce inferior results due to reaction with the resin. Osmium Ammine B at low pH shows a strong specificity for nucleic acids.[21]

K4M and K11M are hydrophilic resins. Therefore, as with other polar (water-miscible) resins, precautions should be taken to ensure that the block face does not become wet during sectioning. Sections should be collected as soon as possible after they are cut and staining incubation should be kept as brief as possible.

Precautions and Storage:

The chemical, physical and toxicological properties of these products are not fully known. Avoid contact with skin and eyes. Avoid inhalation of resin vapor. Use well-ventilated fume hood for mixing resins. Although not as toxic as epoxy resins, it has been shown that methacrylate resins can cause irritation to skin and eyes, and may cause sensitization to some individuals. Vinyl or PVDC gloves are strictly recommended. The use of disposable utensils and tools is recommended.

Note: Kits should **not** be stored in the refrigerator.

In case of contact, prompty wash affected area of skin with plenty of soap and water. Flush eyes with plenty of soap and water. Get medical attention immediately. After handling, wash thoroughly.

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