Lowicryl Embedding Media

#14330, #14340, #14360

Lowicryl® K4M, 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 (references 1-3) . These resins have been formulated to provide low viscosity at low temperature: K4M and HM20 have been developed with freezing points that allows applications downs to about -35°C and -70°C respectively. K11M and HM23 have been developed with freezing points that allow applications down to about -60 °C and -80°C respectively. 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 (360nn) ultraviolet light. Since the initiation of the photopolymerization is largely independent of temperature; block may be polymerized at the same temperatures which are used for infiltration. A rapid polymerization method has also been reported (references 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 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 immunolabeling of sections using specific antisera, lectins, and colloidal gold particles (references 5-8) . The use of K4M or K11M results in a better structural preservation (references 9) , and improved preservation of antigenicity and a significantly lower background labeling (references 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 (references 16) . HM20 and HM23 are particularly suitable for dark-field observation because of their low density compared to conventional embedding media. They can also be used routinely at temperature 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 (references 17) .

Preparation of Lowicryl® Media

The Lowicryl® are provided as highly purified 3-component systems: 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 resins should be prepared in brown glass containers 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 mixture below gives blocks with a medium hardness for Ultraviolet Polymerization:

K4M	HM20	K11M	HM23**
Crosslinker A 2.70g	Crosslinker D 2.98g	Crosslinker 1.0g	Crosslinker 1.1g
Monomer B 17.30g	Monomer 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.*

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

Ethanol, Vol %	Temperature (°C)	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.

Ethanol, vol:vol	Temperature °C	Time
1:1	-35	(-50 to -80)*
2.1	-35	(-50 to -80)*
Pure resin		-35 (-50 to -80)*

** suggested steps for work at 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 still lower temperatures have to be explored. Nothing precise is known as yet about the completeness of dehydration at these two temperatures. Lack of success, due to a late low of water, e.g. in the pyramid of the block, should not automatically be interpreted as a "bad infiltration". The demonstrated persistence of lipids at lower temperatures seems not to cause problems of infiltration (references 19).

Polymerization

The resin is polymerized by direct long-wave length UV-irradiation 360nm 2x15 watt (Philips TLAD 15W/05 or equivalent) at -30°C to -40°C at a distance of 30-40 cm for 24 hours with EMS High Intensity Lamp (100 watt), Cat. #72414, keep distance greater than 85cm). Hardening can be done in filled to capacity gelatine or BEEM® 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, through the sectioning quality improves when they are further hardened under UV light at room temperature for 2-3 days.

Sectioning and Staining

Lowicryl® easily yields silver to gray sections on diamond or glass knives. Optimal sectioning can be done easily with uranyl acetate and Reynold's lead citrate. Oxidizing heavy metal stains like OsO4 or KMnO4 produce inferior results due to the reaction with the resin.

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 contacts 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 PVC gloves are recommended (EMS Cat. #71108). The use of disposable utensils and tools is recommended. Note: Kits should not be stored in the refrigerator.

In case of contact, promptly wash with plenty of soap and water. Flush eyes with plenty of water. Get medical attention immediately.

References

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