

Technical Data Sheet

Unicryl™

#14660

A Universal Resin for Light and Electron Microscopy

UNICRYL is a unique acrylic resin from EMS which has been developed for universal use in both light and electron microscopy. It has the following applications:

- Light Microscopy Electron Microscopy
- Histology Ultrastructure
- Histochemistry Cytochemistry
- Immunohistochemistry Immunocytochemistry
- Immunolabelling Immunolabelling
- In Situ Hybridisation In Situ Hybridisation
- UNICRYL provides optimum sectioning, labelling and staining qualities for studies in
- animal, plant and microbiological tissues.

1. Why is UNICRYL Unique?

The advantages of UNICRYL for staining and labeling lie both in its preservation of tissue structure and its sectioning characteristics such that proteins, nucleic acids and macromolecules are revealed at the surface of the sections for subsequent incubations. The resin preserves these structures with chemically interacting or cross linking with them. UNICRYL is largely hydrophilic, allowing good access to polar(aqueous) solutions and exhibiting a low background staining or labeling from hydrophobic materials. It also minimizes the denaturation of proteins, allowing true antigenic properties to be maintained. Normal counterstaining properties for both EM and LM studies are excellent due to the hydrophilicity of the resin and its homogeneous structure.

The excellent polymerization properties of UNICRYL are due to the fact that all components of the polymer have similar molecular weights, ensuring even penetration into the tissue. In addition, the enhanced labeling, staining and hybridizing qualities arise from the fact that sections are cleaved from the block face, ahead of the knife edge, thus exposing more of the tissue components at the surface. Epoxy resin sections, however, cut straight through the tissue with flat surfaces without regard to the profile of proteins or nucleic acids present.

It is not necessary to exclude air from the resin during polymerization, but as with most resins vials should be sealed to avoid release of harmful vapors.

2. How Is UNICRYL To be Used?

UNICRYL is easy to use. The resin is provided as a single solution and is used in a similar way to the acrylic resins for embedding tissues. Because it is a single solution no mixing is required. The resin has a long shelf life if stored in the cold. It is miscible with alcohols and has a low viscosity even down to -50°C. The resin can be polymerized by heat or by UV irradiation at lower temperatures as described below.

It is recommended that individual small pieces of tissue (0.5mm) are processed in single capped 1ml eppendorf tubes, BEEM® capsules or gelatin capsules (see below) by simple pipetting of solutions into the tubes. The tubes are then closed before polymerization to contain harmful vapors. Larger pieces of tissue may be processed and polymerized in other suitable vials but care must be taken to seal the vials during the polymerization process. Cells in culture may be processed in situ but again the vials should be enclosed during polymerization. Covering the culture dishes with UV transparent glass or plastic during polymerization will reduce the risk of evaporation. UNICRYL interacts with polystyrene and will not polymerize in vials or containers of this material (e.g. polystyrene petri dishes). Polyethylene or glass vials and containers are however, suitable.

3. How Is UNICRYL Polymerized?

The rate of polymerization will determine the cutting properties of a resin. UNICRYL has been designed to have excellent cutting properties when correctly polymerized by heat or UV light. It is important to understand the mechanism of polymerization to achieve the best results.

There are 3 steps involved in the polymerization process. During UV irradiation or heat activation the following steps occur.

- A. Free radicals of initiator are released into the solution. If the temperature is high or if the UV light is very intense the rate of release of free radicals is increased.
- B. The polymer chain begins to grow.
- C. The radicals from the initiator terminate the polymer chain by capping at each end.

If the rate of release of free radicals into solution is too fast the growth of the polymer chain is stopped by early termination. This can be caused by the temperature being too high and or the UV irradiation being too intense. This results in the resin becoming brittle and causing difficulties with cutting.

If the release of radicals from the initiator into solution is too slow then the polymer chain does not grow quickly but there is also a low rate of termination of the chain by the radicals. The resin then takes a long time to fully polymerize. Lower temperatures lengthen the polymerization time unless the illumination is increased (see table below).

3.1 Polymerization By Heat or UV Irradiation:

Polymerization of resins is an exothermic reaction and may cause the temperature in the tissue block to rise. It may therefore be necessary to control the temperature by performing the polymerization reaction in a cold chamber or surrounding the tissue with a suitable heat sink (see below). Polymerization may take place by input of energy by two different methods. The resin may (a) be heated or, (b) it may be irradiated with UV light of an appropriate wavelength. UV irradiation also causes a rise in temperature which in turn also produces polymerization. UV light should therefore be used under controlled low temperatures if high temperature is likely to interfere with the specimen antigens or structure. UNICRYL shrinks by approximately 10% in volume during polymerization. Evaporation may be controlled by using enclosed vials or covered molds at elevated temperatures. Please inquire.

3.2 Choice of polymerization temperature

A choice must be whether to polymerize the resin at high or low temperatures. If the antigens or tissue components are sensitive to temperature rises then cooling methods should be employed with UV irradiation polymerization. For all other cases where temperature rises up to 60°C are not important, simple heat polymerization may be used without UV irradiation.

Although low temperature dehydration and/or freeze substitution can be used for infiltrating tissue with resin, it is not usually necessary to cool the tissue and resin to very low temperatures for the polymerization step. Freeze substitution may be performed at -50°C in order to preserve soluble components of the tissue but final polymerization may still be satisfactory at 4°C or even higher according to the requirements of the tissue. In most living tissues organic reactions occur at ambient temperatures or above so the need for low temperature polymerization is not generally required. There is also some evidence to show that very low temperatures may cause loss of antigenicity through denaturing of the antigen. The main reason for maintaining the specimen at low temperature (e.g. 4 °C) during polymerization is to ensure that the exothermic reaction during the polymerization does not cause the specimen temperature to rise above 37°C.

3.3 Polymerization at High Temperature:

This method may be used where tissues are not temperature sensitive. High temperature polymerization (e.g. 50-60°C) will be acceptable for most histological and ultrastructural studies where antigenicity is not important. Even for many immunolabelling methods and in situ hybridization techniques the high temperatures may not affect the results but should be made with tissues polymerized at low temperatures (see below).

It should be remembered that the exothermic nature of the reaction will add to the specimen temperature rise and this should be taken into account when setting the temperature of the oven. In order to minimize the temperature rise it is recommended that the minimum amount of resin is used to embed the specimen during the polymerization step. Use only enough resin to embed and surround the tissue for subsequent

handling since larger volumes of resin will produce larger exothermic reactions and thus a greater temperature rise.

3.3.1 Typical Protocol for High Temperature Polymerization:

- A. Fix the tissue appropriately for EM and LM applications.
- B. Wash thoroughly in buffer. Dehydrate in 70%, 90%, 100% ethyl alcohol, acetone, or other dehydrating agents as appropriate (typically 3x10min).
- C. Infiltrate with 100% resin, 2x1h, while agitating gently on a shaker or rotating wheel. Use a ratio of 100 x the tissue volume (e.g. 1mm³ requires 1ml).
- D. Infiltrate with fresh resin for at least 8h while gently agitating, preferable overnight to allow full penetration before polymerization. Use the minimum possible volume of resin for convenient block handling.
- E. Place the vials in a temperature controlled oven. Typically polymerize for 1-2 days in 60°C or longer at 50°C (see below).

All the above procedures can be performed in disposable glass or low density polyethylene or polypropylene vials using 100x the total tissue volume. For single pieces of tissue use 1ml eppendorf centrifuge tubes or other low density polyethylene vials. Vials with snap closing lids are useful to allow agitation during the infiltration of the tissue (although it is not necessary to exclude oxygen during the polymerization).

Other suitable vials are BEEM® or gelatin capsules for small pieces of tissue. Some control samples of resin should be used to judge the optimal polymerization time and temperature for best cutting characteristics. In order to avoid an excessive rise in temperature during polymerization from the exothermic reaction, a metal (e.g. aluminum) heat sink may be used. The metal block should be drilled with holes to accommodate the vials in order to disperse any excessive heat.

3.3.2 Typical Heat Polymerization Times For UNICRYL:

Temperature Time for polymerization (1ml)

50°C 2-3 days

60°C 1-2 days

70°C 1 day (brittle)

Larger blocks will polymerize more rapidly due to greater exothermic reactions.

3.4 Polymerization By UV Light:

UV light polymerization should be used for tissues which are temperature sensitive. UV light may be used at any temperature from -10°C to +20°C. The lower the temperature the longer the polymerization reaction

takes unless the light intensity is adjusted. The resin is designed to be perfectly polymerized after 2-3 days with UV light of 2x8 watts held at 15cm from the specimen 4°C. If the temperature is lower than 4°C the light intensity can be increased by reducing the specimen-lamp distance. It is preferable to adjust the light intensity to achieve polymerization in 2-3 days for the reasons given above. Trial samples of resin can be used to achieve the best conditions (see table below).

3.4.1 Polymerization Chamber:

A simple portable chamber may be constructed from a box. 2x8w UV strip lights may be wired to a plug outside the chamber. The box may be easily removed from fume hood to refrigerator or freezer as required. The UV source should emit light at 360nm (long wavelength). Suitable types of lamps are the Philips TL8w/05.

3.4.2 Light Arrangements For UV Polymerization:

A) Direct or indirect light:

It is important to understand that indirect light may be less intense than direct light. The total distance that the light travels is important in determining the final intensity. The intensity of light falls off as the square of the distance (inverse square law). Thus light at 30 cm is 1/4 as intense as light at 15cm, $(15/30)^2$, while light at 5cm is 9x as intense as light at 15cm, $(15/5)^2$. Therefore if the sample is irradiated indirectly with UV light in a chamber, with the light being reflected compared with direct illumination, in addition, some light absorption occurs on the chamber walls, reducing the total intensity at the specimen.

It is recommended that the specimen is illuminated from below in the case of tissue in vials (see diagram above). This will allow the minimum amount of resin between the specimen and the light source. If the specimen is illuminated through a large amount of resin, i.e., from above, then the light intensity is reduced at the tissue and local polymerization at the tissue takes longer. It sometimes found that when illuminated from below, the tissue is polymerized first and the resin at the top of the vial remains liquid for a longer period.

When polymerizing tissue in open dishes, such as in embedded cultures, it is preferable to irradiate from above to prevent absorption of light at the base of the dish. When polymerizing tissue pieces in a plastic embedding mold, irradiation should be from above for the same reasons. Such molds and dishes should be covered with UV transparent glass or plastic coverslips to reduce evaporation. Special "PTFE" molds are available for polymerization of resin blocks. Aluminum foil molds can also be made.

B) UV Light Intensity:

The recommended light intensity for polymerizing UNICRYL at 4°C is given by 2x8 watt lamps (long wavelength) at a distance of 15cm. This will produce satisfactory polymerization in 2-3 days (depending on the quantity of resin in the vial) and produces excellent cutting properties. With this arrangement at 20°C

polymerization may take only 1-2 days (see below). If lower power lamps are used, e.g. 2x6 watts then the total intensity is reduced by the ratio of the power. This can be compensated for by reducing the distance from lamp to specimen, using the inverse square law as described above. For example, changing from 2x8 watts to 2x6 watts will require reducing the specimen lamp distance from 15cm to approximately 12 cm to achieve polymerization in the same time. Otherwise the time for polymerization will be lengthened. Similarly, increasing the illumination from 2x8 watts to 2x15 watts will double the intensity and may require increasing the specimen-lamp distance or using a diffusing screen to avoid too rapid polymerization.

C) Wavelength Of UV Light:

The wavelength of the light to be used is also important for both polymerization and tissue integrity. The longer the wavelength the lower the energy. The recommended wavelength for polymerization is 360nm. Lamps suitable for polymerization are those such as used for thin layer chromatography. If a lamp is used with both long and short wavelength UV light it is necessary to mask the short wavelength with a filter to prevent too high energy light being used and interfering with the polymerization of the resin and with the tissue antigens and nucleic acids. If the wavelength is too long (i.e. lower energy) then the polymerization may not be stimulated.

D) Polymerization of Control Samples of Resin:

If UV light is being used for polymerization it is recommended that some resin is also pipetted into an extra 1ml vial and heated in a 60°C oven for 2 days to demonstrate the satisfactory nature of the polymerization. In addition, some control vials of resin without tissue can be polymerized at low temperatures with various arrangements of UV light to determine the optimum conditions for polymerization of tissue.

E) Polymerization With Stained or Colored Tissue:

Tissues that are heavily stained, such as those fixed in osmium tetroxide or picric acid, become colored and will absorb light more readily. Best immunolabeling results are usually obtained without the use of osmium tetroxide. As an alternative to osmium tetroxide for membrane fixation, tannic acid has been used successfully (4). Some tissues are also more heavily pigmented than others and may more readily absorb UV light. There may be a local rise in temperature within the resin that can cause the tissue to polymerize more quickly than the rest of the surrounding resin. This may result in brittleness of the tissue block and a decrease in antigenicity. It is, however possible to overcome this problem by ensuring that the tissue block does not rise in temperature during UV irradiation by surrounding the tissue and resin with a suitably cooled metal heat sink during the irradiation and performing the polymerization in a cold chamber as described below. Alternatively the irradiation can be reduced by increasing the specimen-lamp distance.

3.4.3 Typical UV Polymerization Times For UNICRYL:

Temperature Specimen-lamp distance (cm for 2x8w lamps (direct illumination))

Temp	1cm	5cm	10cm	15cm	20cm
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+20°C	brittle	brittle	brittle	1-2 days	2-3 days
+4°C	brittle	brittle	1-2 days	2-3 days	3-4 days
-10°C	1-2 days	2 days	2-3 days	3-4 days	4-5 days

Times given are approximate and depend on the quality of resin, opacity of the vials, and the efficiency of the lamps at different temperatures.

3.4.4 Typical Protocol For UV Polymerization:

- A. Process the tissue as for heat polymerization (3.3.1 above) but if possible avoiding the use of coloring fixes such as osmium tetroxide or picric acid (see above). Use tannic acid fix as an alternative if necessary (4).
- B. Place the vials of tissue in an appropriate temperature controlled UV chamber.
- C. Irradiate of UV light for the appropriate time as described. The vials may be examined periodically to determine their hardness. Extra control vials of resin may be taken out of different time intervals to test for cutting quality. The embedded tissue may be left in the UV chamber for extended lengths of time without excessive polymerization occurring.

3.4.5 Progressive Lowering of Temperature (PLT):

UNICRYL will remain liquid to -50°C. It is therefore possible to dehydrate the tissue in alcohol while progressively lowering the temperature. Therefore the tissue may be embedded with resin at low temperature and subsequently polymerized at any other chosen temperature. This technique is of value where specimens are particularly sensitive to alcohol at ambient temperatures. It is not always necessary to polymerize the resin at the same low temperature. Normally the resin can be polymerized at 4 degrees C to avoid temperature rises during exothermic reactions. Polymerization of UNICRYL at lower temperatures takes longer unless UV irradiation conditions are adjusted (see table above).

A Typical PLT Procedure Is As Follows:

1. Fix the tissue appropriately for EM or LM applications.
2. Wash thoroughly in buffer.
3. Dehydrate in increasing alcohol concentration (30, 50, 70, 95, 100%)(1h each) while reducing the
4. temperature at each stage. Pure ethyl alcohol will remain liquid in -50C
5. Infiltrate with 100% resin at -30°C (2x1h). If possible while gently agitating.
6. Infiltrate with fresh resin at -30°C for at least 8h, preferably overnight, if possible while gently agitating.
7. Irradiate with UV light at the chosen temperature and for the appropriate time as described. The vials may be examined periodically to determine their hardness. Extra control vials of resin may be taken out at different time intervals to test for cutting quality. The embedding tissue may be left in the UV chamber for extended lengths of time without excessive polymerization occurring.

4. What Are The Cutting Properties of UNICRYL?

When fully and carefully polymerized the resin will demonstrate excellent cutting characters without ripples or bubbles. If these occur the most likely cause will be inadequate infiltration and embedding before polymerization, or inadequate polymerization time. Bubbles may also occur if polymerization has been too rapid (see below). This is more likely to happen in larger blocks where the increased exothermic reaction speeds up the polymerization at the center of the block.

The section surface follows the contours of the tissue during the cutting action, effectively cleaving the section from the block ahead of the knife edge. This produces a highly exposed surface of proteins and nucleic acids for subsequent access to incubating solutions of antibodies, stains and probes.

For LM studies, sections from 0.2-2µm are easily obtained with glass or diamond knives. For larger specimens (e.g. >3mm) a glass Ralph knife may be used for best results. The sections may be floated on drops of water onto slides coated with BIOBOND for maximum retention during incubations. The sections adhere strongly when dried onto the coated slides.

For EM studies sections ranging from gray to green interference colors (>0.1 - 0.2µm) are also readily produced, especially with a diamond knife. The sections have good stability in the electron beam but may also be supported on collodion, formvar or carbon films if preferred.

The cutting properties of the resin should not be judged during the initial trimming of the block with a razor blade. When properly polymerized the resin will cut sections evenly with a sharp glass or diamond knife even if initial trimming with a razor blade gives a brittle appearance.

5. What Are The Labeling and Staining Characteristics of UNICRYL?

Because of the exposure of proteins and nucleic acids at the section surface, UNICRYL exhibits strong labeling and staining properties. In addition the resin is hydrophilic and readily wets with aqueous solutions.

For immunolabelling, UNICRYL exhibits high specificity with virtual absence of background staining. Ultrastructural preservation is excellent and stability maintained in the EM without carbon coating.

For routine histology polychromatic stains are readily absorbed, while for EM the normal heavy metal counterstains work without difficulty and more quickly than for epoxy resins. This is due to the regular molecular weight distribution of the monomers employed in the resin design, giving uniform tissue penetration and section surface exposure of proteins. In addition, the close mesh structure helps to produce good electron beam stability.

For in situ hybridization identification of nucleic acid sequences is optimized because of their accessibility at the resin surface. At both LM and EM levels the sections show good stability under hybridization conditions with minimal background labeling.

6. Troubleshooting:

UNICRYL is easy to use and presents relatively few problems. The following, however, are typical questions that may arise during use of any acrylics.

Q1. The resin does not polymerize fully in two days.

The combination of temperature and light intensity is incorrect (see above). Colder temperatures require higher light intensity and longer times. The times given in the table above are approximate and depend upon the quantity of resin in the vial, the opacity of the vial, and the performance of the lamps at low temperature. Some UV lamps may be less effective at lower temperatures.

Q2. *Part of the resin is polymerized and part remains liquid.*

- A. UV light is prevented from fully reaching the whole of the resin. Irradiate the tissue directly if possible. Use reflecting metal foil to surround the block in order to irradiate from all sides.
- B. Prolong the UV irradiation.

Q3. *Bubbles appear in the polymerized resin block.*

- A. The polymerization has been too rapid or the temperature is too high. Reduce the temperature and polymerize again with fresh resin. Test conditions for polymerization with a sample resin block. Use the minimum possible volume of resin. Larger blocks cause greater exothermic reactions and higher temperature rises.
- B. The tissue was inadequately infiltrated with resin before polymerization. Embed for longer.
- C. Air was trapped in the resin by too severe agitation while embedding.

Q4. *Sections show ripples.*

- A. The block is not fully polymerized.
- B. The block is loose in the chuck.
- C. The knife is blunt/loose.

Q5. *Sections dissolve on the water bath.*

The block is not fully polymerized.

Q6. *The block is brittle.*

Polymerization has been too rapid. Adjust the temperature/distance/light intensity as described above to give full polymerization in two days or longer.

Q7. Holes appear in sections. Tissue ultrastructure is poor.

- A. Fixation of the tissue is inadequate.
- B. Dehydration of the tissue has extracted some components.
- C. Penetration of the tissue by the resin has been incomplete. Lengthen the resin infiltrating and embedding steps to allow full exchange with the dehydrating agent used (e.g. alcohol). Infiltrate for at least 8 hours before polymerizing.
- D. Polymerization was too rapid (too hot), producing bubbles in the tissue. Use less resin and longer polymerization.

Q8. Sections are unstable in the electron beam.

- A. The resin has not been fully polymerized.
- B. The electron beam is too strong. If necessary use plastic support films.

Q9. Labeling is poor.

- A. Tissue fixation is harmful to antigens. Carefully select the optimum fixation schedule. Avoid the use of osmium tetroxide if possible.
- B. Polymerization method is inadequate. Antigens may be sensitive to high temperature. Compare low temperature polymerization method.
- C. Antigens are sensitive to low temperatures. Compare dehydration and polymerization at 20°C.
- D. Antigens are affected by UV irradiation. Compare thermal polymerization.

Q10. Resin hardness varies throughout the tissue.

Polymerization is not complete. Continue to polymerize until even section cutting is obtained.

Q11. Resin was left to polymerize for an excessive time. Will it be damage?

It is not possible to over-polymerize UNICRYL with prolonged exposure to light or heat. It is important, however, to consider the effect of continuous UV or high temperature on antigenicity of the tissue.

Q12. Approximately 10% shrinkage in volume occurs during the polymerization step due to the crosslinking of the polymer. In addition, some plastic materials are porous to the acrylics in the liquid state, giving the appearance of loss of resin. Some evaporation may occur if the temperature is above 60°C. Use sealed vials and cover embedding molds and culture dishes with UV transparent coverslips.

7. Storage of UNICRYL

UNICRYL can be stored at 4°C for at least 12 months. It will not freeze at temperatures above -50°C and can be used from the refrigerator daily. It should be kept in the dark plastic bottle provided.

Small pieces of dehydrated tissue can be successfully stored in unpolymerized UNICRYL at 4°C or -20°C until needed for polymerization.

8. Safety and handling (see hazard information sheets provided)

Most embedding media are hazardous to a certain extent and may cause reactions on contact with skin. UNICRYL is a methacrylate based resin and has components listed in Appendix VI of German Hazardous Substances Ordinance. Methacrylates may cause some allergic responses and should be handled accordingly.

All handling of UNICRYL should be performed under a fume hood while wearing rubber gloves, laboratory coat and eye protection as normal. Once the resin is in sealed eppendorf tubes it may be handled out of the fume hood (e.g. in a refrigerator or freezer). Avoid breathing the vapor. The resin produces a recognizable pungent odor.

Excess resin must be decanted or pipetted into a clear plastic bottle, sealed and polymerized with UV light as below. Spilled resin, contaminated vials, and used rubber glove should be absorbed with tissue paper, sealed in a clear plastic bag (PVC or polyethylene) and polymerized to render it harmless. It may then be disposed of through normal laboratory chemical waste.

9. Questions About UNICRYL:

Q1. What is so new about UNICRYL?

UNICRYL is an easy to use resin which gives excellent structural preservation of tissue together with highly efficient labelling and staining for both LM and EM. It is the only single solution all purpose resin that can be used over such a wide range of polymerising temperatures and for so many different applications. It is also extremely stable in the electron beam.

Q2. Why does UNICRYL provide these good labelling properties and structural preservation?

The components of UNICRYL penetrate the tissue easily and evenly and retain tissue components in situ. The polymerized resin is largely hydrophilic and presents a highly exposed surface in thin sections. The revealed tissue components are then readily accessible to the staining and incubating solutions, antibodies and nucleic acid probes.

Q.3 How easy is it to use?

UNICRYL is a single solution that is readily miscible with most dehydrating agents and has a low viscosity even at -50°C. It is simply pipetted into vials containing tissue pieces for polymerisation by heat or UV light at low temperatures.

Q.4. Can UNICRYL be used for any tissue?

UNICRYL can be used for embedding plant, animal, and microbiological tissues and cells. It is suitable for both hard and soft tissues. It can be infiltrated at temperatures down to -50°C where it is still a liquid.

Q.5 How is UNICRYL supplied?

UNICRYL is supplied in 250 ml volumes. Each UNICRYL Kit is provided with a pack of 1 ml eppendorf vials for embedding tissue, a set of plastic gloves, plastic bags for disposal, and a container for waste resin. The UNICRYL Embedding Kit (EMS Catalog #14660) can be obtained from Electron Microscopy Sciences.

Please feel welcome to contact the Technical Services Department at Electron Microscopy Sciences for further discussion about UNICRYL or any other aspect of immunolabelling. If you would like information about other products manufactured by British BioCell International, please ask for your copy of the catalogue from Electron Microscopy Sciences.

10. Staining UNICRYL sections with the UNICRYL Staining Kit.

Sections cut from UNICRYL embedded tissues reveal proteins and nucleic acids more readily at the surface than in epoxy resins. The UNICRYL Staining Kit (EMS Catalog #14960) provides a set of 6 stains (100ml each) sufficient for polychromatic staining of over 100 sections of glass slides. Using this Staining brilliant results are obtained in a very short staining time. The Kit contains 100ml volumes of haematoxylin, silver methenamine, eosin, fast green, toluidine blue, and safranin, all specially prepared for optimum staining of UNICRYL embedded sections. Detailed instructions on staining protocols are included.

11. UV polymerisation lamps

Ultraviolet polymerising lamps are available for use in the construction of a polymerisation chamber. These are provided as a pair of 8w UV lamps of 360nm wavelength in 12" fittings ready for wiring to a 240V source. For other voltages a suitable adaptor is required.

12. References

1. Scala C, Cenacchi G, ferrari C, Pasquinelli G, Preda P, Manara GC (1992) A new acrylic resin formulation/: A useful tool for histological, ultrastructural and immunocytochemical investigations. *J Histochem Cytochem* (1992), 40(11), 1799-180?
2. Cenacchi G, Musiani M, Gentilomi G, Zerbini M, Chandler J, Scala C, Martinelli G (1992). In situ hybridisation at ultrastructural level: Localisation of viral DNA using digoxigenin labelled probes. 10th European Congress on Electron Microscopy, Granada, Sept. 1992, abstract p167.
3. Scala C, Badiali DeGiorgi L, Cenacchi G, Preda P, Vici M, Pasquinelli G (1992). Development of a new acrylic resin ideally suited for light and electron microscopy. 10th European Congress on Electron Microscopy, Granada, Sept. 1992, abstract p271.

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

