BBI Solutions™

UNICRYL™

A Universal Resin for Light and Electron Microscopy

UNICRYL is a unique acrylic resin from BBI Solutions 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.

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1. Why is UNICRYL unique?

The advantages of UNICRYL for staining and labelling lie in both its preservation of tissue structures 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 without 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 labelling from hydrophobic materials. It also minimises 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 hydrophobicity of the resin and its homogeneous structure (1,2,3).

The excellent polymerisation properties of UNICRYL are due to the fact that all the components of the polymer have similar molecular weights, ensuring even penetration into the tissue. In addition, the enhanced labelling, staining and hybridising qualities arise from the fact that sections are cleaved from the block face, ahead of the knife edges, 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 polymerisation, but as with most resins vials should be sealed to avoid release of harmful vapours.

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 other 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 -50C, The resin can be polymerised by heat or by UV irradiation at lower temperatures as described below.

It is recommended that individual small pieces of tissue (0.5mm3) 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 polymerisation to contain harmful vapours. Larger pieces of tissue may be processed and polymerised in other suitable vials but care must be taken to seal the vials during the polymerisation process. Cells in culture may be processed in situ but again the vials should be enclosed during polymerisation. Covering the culture dishes with UV transparent glass or plastic during polymerisation will reduce the risk of evaporation.

UNICRYL interacts with polystyrene and will not polymerise in vials or containers of this material (eg polystyrene petri dishes). Polyethylene or glass vials and containers are, however, suitable.

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3. How is UNICRYL polymerised?

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

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

a) Free radicals of the indicator are released into 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 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 polymerise. Lower temperatures lengthen the polymerisation time unless the intensity of illumination is increased (see table below).

3.1 Polymerisation by heat or UV irradiation

Polymerisation 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 polymerisation reaction in a cold chamber or surrounding the tissue with a suitable heat sink (see below). Polymerisation 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 polymerisation. 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 polymerisation. Evaporation may be controlled by using enclosed vials or covered moulds at elevated temperatures.

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3.2 Choice of polymerisation temperature

A choice must be made whether to polymerise 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 polymerisation. For all other cases where temperature rises up to 60C are not important, simple heat polymerisation 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 polymerisation step.** Freeze substitution may be performed at -50C in order to preserve soluble components of the tissue but final polymerisation may still be satisfactory at 4C 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 polymerisation 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 (eg 4C) during polymerisation is to ensure that the exothermic reaction during the polymerisation does not cause the specimen temperature to rise above 37C.

3.3 Polymerisation at High Temperature

This method may be used where tissues are not temperature sensitive. High temperature polymerisation (eg 50-60C) will be acceptable for most histological and ultrastructural studies where antigenicity is not important. Even for many immunolabelling methods and in situ hybridisation techniques the high temperatures may not affect the results but comparisons should be made with tissues polymerised 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 minimise the temperature rise it is recommended that the minimum amount of resin is used to embed the specimen during the polymerisation 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.

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3.3.1 Typical protocol for high temperature polymerisation

1. Fix the tissue appropriately for EM or LM applications.
2. Wash thoroughly in buffer.
3. Dehydrate in 70%, 90%, 100% ethyl alcohol, acetone, or other dehydrating agents as appropriate (typical 3x10 min).
4. Infiltrate with 100% resin, 2x1h, while agitating gently on a shaker or rotating wheel. Use a ratio of 100x the tissue volume (eg 1mm3 requires 1ml).
5. infiltrate with fresh resin for at least 8h while gently agitating, preferably overnight to allow full penetration before polymerisation. Use the minimum possible volume of resin for convenient block handling.
6. Place the vials in a temperature controlled oven. Typically polymerise for 1-2 days at 60C or longer at 50C (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 polymerisation).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 polymerisation time and temperature for best cutting characteristics. In order to avoid an excessive rise in temperature during polymerisation from the exothermic reaction, a metal (eg aluminium) 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 polymerisation times for UNICRYL

Temperature	Time for polymerisation (1ml)
50C	2-3 days
60C	1-2 days
70C	1 day (brittle)

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

3.3.3 Modified Procedure

Bogers et al 1996 suggested that improved results could be obtained when preparing tissues for electron microscopy using UNICRYL. The basic protocol for infiltrating, embedding and polymerising UNICRYL as described in the instruction booklet remain unchanged. Users may like to consider the small modifications proposed by the authors of this publication to enhance the ultrastructural preservation of some tissues and cells in general or in more difficult situations. Basically the suggested improvements are to increase the extent of the infiltration of tissue as follows:

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Basic protocol 3.3.1 (page 5 of the instruction Booklet)

1. Fix the tissue appropriately.
2. Wash thoroughly in buffer.
3. Dehydrate in 70%, 90%, 100% ethyl alcohol, acetone or other dehydrating agent as appropriate (typically 3x10 min)
4. Infiltrate with 100% resin, 2x1hr, while agitating gently on a shaker or rotating wheel.
5. Infiltrate with fresh resin for at least 8h while gently agitating, preferably overnight to allow full penetration before polymerisation. Use the minimum possible volume of resin for convenient block handling.
6. Place the vials in a temperature controlled oven. Typically polymerise for 1-2 days at 60°C or longer at 50°C.

Modified Procedure

1. As above.
2. As above.
3. As above

3a. Infiltrate with 1:2 UNICRYL: ethanol for 30 minutes

3b. Infiltrate with 2:1 UNICRYL: ethanol for 30 minutes

4. As above
5. As above
6. As above

The same additional steps may be incorporated into the low temperature embedding procedure described on page 10 of the Instruction Booklet.

3.4 Polymerisation by UV Light

UV light polymerisation should be used for tissues which are temperature sensitive. UV light may be used at any temperature from -10C to +20C.
The lower the temperature the longer the polymerisation reaction takes unless the light intensity is adjusted. The resin is designed to be perfectly polymerised after 2-3 days with UV light of 2x8 watt held at 15cm from the specimen at 4C.

If the temperature is lower than 4C the light intensity can be increased by reducing the specimen-lamp distance and/or by increasing the illumination power (wattage). If the temperature is higher than 4C then the light intensity can be decreased by increasing the specimen-lamp distance. it is preferable to adjust the light intensity to achieve polymerisation in 2-3 days for the reasons given above. Trial samples of resin can be used to achieve the best conditions.

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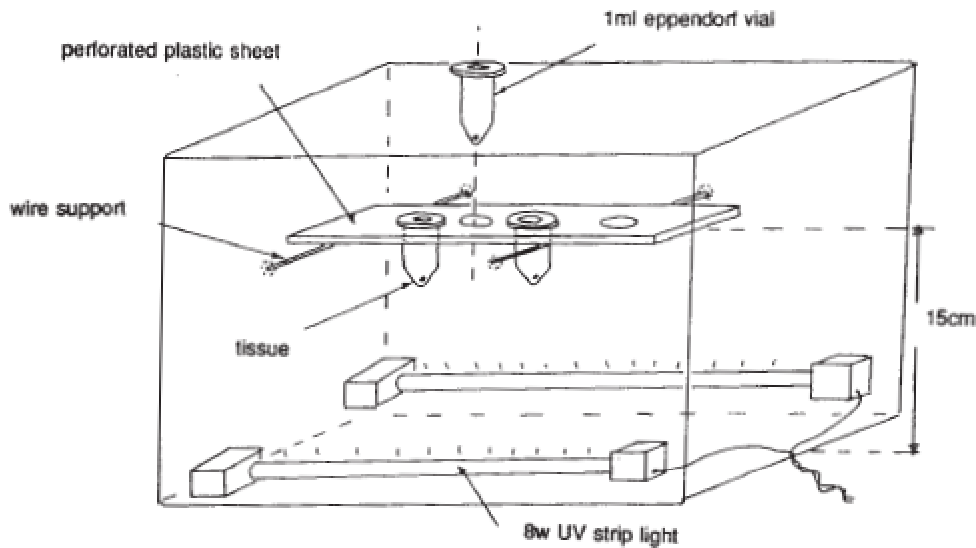

3.4.1 Polymerisation chamber

A simple portable chamber may be constructed from a box as shown below. 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 TL 8w/05. Hand held UV lamps and low temperature UV polymerisation chambers are also supplied by Agar Scientific Ltd (UK).

3.4.2 Light arrangements for UV polymerisation

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 30cm 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 from the chamber walls, the total illumination intensity may be much reduced 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, ie from above, then the light intensity is reduced at the tissue and local polymerisation at the tissue takes longer. It is sometimes found that when illuminated from below, the tissue is polymerised first and the resin at the top of the vial remains liquid for a longer period.

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When polymerising 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 polymerising tissue pieces in a plastic embedding mould, irradiation should be from above for the same reasons. Such moulds and dishes should be covered with UV transparent glass or plastic coverslips to reduce evaporation. Special PTFE moulds are available for polymerisation of resin blocks. Aluminium foil moulds can also be made.

b) UV Light intensity

The recommended light intensity for polymerising UNICRYL at 4C is given by 2x8 watt lamps (long wavelength) at a distance of 15cm. This will produce satisfactory polymerisation in 2-3 days (depending on the quantity of resin in the vial) and produces excellent cutting properties. With this arrangement at 20C polymerisation may take only 1-2 days (see below). If lower power lamps are used, eg 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 12cm to achieve polymerisation in the same time. Otherwise the time for polymerisation 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 polymerisation.

c) Wavelength of UV light

The wavelength of the light to be used is also important for both polymerisation and tissue integrity. The longer the wavelength the lower the energy. The recommended wavelength for polymerisation is 360nm. Lamps suitable for polymerisation 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 polymerisation of the resin and with the tissue antigens and nucleic acids. If the wavelength is too long (ie lower energy) then the polymerisation may not be stimulated.

d) Polymerisation of control samples of resin

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

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e) Polymerisation with stained or coloured tissue

Tissues that are heavily stained, such as those fixed in osmium tetroxide or picric acid, become coloured and will absorb light more readily. Best immunolabelling 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 polymerise more quickly that 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 bock 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 polymerisation in a cold chamber as described below. Alternatively the irradiation can be reduced by increasing the specimen-lamp distance.

3.4.3 Typical UV polymerisation times for UNICRYL

Temp	Specimen-lamp distance (cm) for 2x8w lamps (direct illumination)				
	1cm	5cm	10cm	15cm	20cm
+20C	brittle	brittle	brittle	1-2 days	2-3 days
+4C	brittle	brittle	1-2 days	2-3 days	3-4 days
-10C	1-2 days	2 days	2-3 days	3-4 days	4-5 days

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

3.4.4 Typical protocol for UV polymerisation

1. Process the tissue as for heat polymerisation (3.3.1 above) but if possible avoiding the use of coloured fixes such as osmium tetroxide or picric acid (see above). Use tannic acid fix as an alternative if necessary (4).
2. Place the vials of tissue in an appropriate temperature controlled UV chamber.
3. Irradiate with 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 at 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 polymerisation occurring.

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3.4.5 Progressive lowering of temperature (PLT)

UNICRYL will remain liquid to -50C. It is therefore possible to dehydrate the tissue in alcohol while progressively lowering the temperature. Thereafter the tissue may be embedded with resin at low temperature and subsequently polymerised 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 polymerise the resin at the same low temperature.** Normally the resin can be polymerised at 4C to avoid temperature rises during exothermic reactions. Polymerisation 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 temperature at each stage. Pure ethyl alcohol will remain liquid to -50C.
4. Infiltrate with 100% resin at -30C (2x1h), if possible while gently agitating.
5. Infiltrate with fresh resin at -30C for at least 8h, preferably overnight, if possible while gently agitating.
6. 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 embedded tissue may be left in the UV chamber for extended lengths of time without excessive polymerisation occurring.

4. What are the current properties of UNICRYL?

When fully and carefully polymerised the resin will demonstrate excellent cutting characteristics without ripples or bubbles. If these occur the most likely cause will be inadequate infiltration and embedding before polymerisation, or inadequate polymerisation time. Bubbles may also occur if polymerisation has been to rapid (see below). This is more likely to happen in larger blocks where the increased exothermic reaction speeds up the polymerisation at the centre 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 0.5-2µm are easily obtained with glass or diamond knives. For larger specimens (eg>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 (see BBI Solutions Catalogue) for maximum retention during incubations. The sections adhere strongly when dried onto the coated slides.

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For EM studies sections ranging from grey to green interference colours (>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 colloid on, 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 polymerised 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 labelling and staining characteristics of UNICRYL?

Because of the exposure of proteins and nucleic acids at the section surface, UNICRYL exhibits strong labelling 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. Ultra-structural 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 hybridisation identification of nucleic acid sequences is optimised because of their accessibility at the resin surface. At both LM and EM levels the sections show good stability under hybridisation conditions with minimal background labelling.

6. Trouble shooting

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 polymerise 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 efficient at lower temperatures.

Q2. Part of the resin is polymerised and part remains liquid.

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

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Q3. Bubbles appear in the polymerised resin block.

a) The polymerisation has been too rapid or the temperature too high. Reduce the temperature and polymerise again with fresh resin. Test conditions for polymerisation 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 polymerisation. 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 polymerised.
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 polymerised.

Q6. The block is brittle.

Polymerisation has been too rapid. Adjust the temperature/distance/light intensity as described above to give full polymerisation 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 (eg alcohol). Infiltrate for at least 8 hours before polymerising.
d) Polymerisation was too rapid (too hot), producing bubbles in the tissue. Use less resin and longer polymerisation.

Q8. Sections are unstable in the electron beam.

a) The resin has not been fully polymerised.
b) The electron beam is too strong. If necessary use plastic support films.

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Q9. Labelling is poor.

a) Tissue fixation is harmful to antigens. Carefully select the optimum fixation schedule. Avoid the use of osmium tetroxide if possible.
b) Polymerisation method is inadequate. Antigens may be sensitive to high temperature. Compare low temperature polymerisation method.
c) Antigens are sensitive to low temperatures. Compare dehydration and polymerisation at 20C.
d) Antigens are affected by UV irradiation. Compare thermal polymerisation.

Q10. Resin hardness varies throughout the tissue.

Polymerisation is not complete. Continue to polymerise until even section cutting is obtained.

Q11. Resin was left to polymerise for an excessive time. Will it be damaged?

It is not possible to over-polymerise 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. Resin appears to evaporate.
Approximately 10% shrinkage in volume occurs during the polymerisation Step due to the cross linking 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 60C. Use sealed vials and cover embedding moulds and culture dishes with UV transparent coverslips.

7. Storage of UNICRYL

UNICRYL can be stored at 4C for at least 12 months. It will not freeze at temperatures above -50C 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 unpolymerised UNICRYL at 4C or -20C until needed for polymerisation.

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 the German Hazardous Substances Ordinance. Mathacrylates may cause some allergic responses and should be handled accordingly.

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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 (eg in a refrigerator or freezer). Avoid breathing the vapour. The resin produces a recognised pungent odour.

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

9. Questions about UNICRYL

Q1. What is so good about UNICRYL?

A. 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?

A. The components of UNICRYL penetrate the tissue easily and evenly and retain tissue components in situ. The polymerised 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.

Q3. How easy is it to use?

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

Q4. Can UNICRYL be used for any tissue?

A. 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 -50C where it is still liquid.

Q5. How is UNICRYL supplied?

A. UNICRYL is supplied in 250ml volumes. The UNICRYL Embedding Kit can be obtained from your local BBISolutions distributor. **Product code: BA250.**

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10. UV polymerisation lamps

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

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UNICRYL™ – Extra Technical Notes

1. Flat embedding

Flat embedding moulds may be used to embed tissues with UNICRYL. Only certain moulds are suitable. Those using hard plastic such as polypropylene are most suitable. They are available from most EM suppliers. The softer rubber and softer plastics may not be suitable. This is because some of the resin components penetrate into the moulds and prevent the resin from polymerising. When using flat embedding moulds at high temperatures (ie 55-60C) the moulds should be covered to prevent undue evaporation from the large surface area. A greased coverslip may be suitable but a tight seal is preferred. A further problem may arise from capillary action along the coverslip, drawing the low viscosity resin away from the well. Wells in the mould may empty by capillary action and should not be over filled initially. When using the flat moulds for UV polymerisation at lower temperatures (eg 4C - 20C) evaporation will not occur so readily and the moulds may not need to be covered. The UV light should be directed from above directly into the resin since most plastics will obscure some of the light. Some clear moulds (eg from SPI Inc) are partially transparent to UV light and may be irradiated from below.

2. Capsule embedding

EM capsules, gelatin capsules and eppendorf type capsules are all suitable. Most polypropylene plastics are impermeable to the resin. It is not necessary to exclude oxygen from the surface of the resin during polymerisation. It is, however, advisable to seal capsules during thermal polymerisation at 55-60C for two reasons. Firstly the resin vapour should be contained for health reasons. Secondly, evaporation of the resin, while not as great as in flat embedding moulds, may still occur to a small extent.

3. Volume of resin

Whether using capsules or mould it is advisable to use the smallest volume of resin possible. This is for two reasons. If using UV polymerisation the outer part of the resin will polymerise first. This slows down the polymerisation of the centre due to attenuation of light. Smaller blocks will therefore polymerise more evenly. Secondly, the exothermic reaction is greater for larger volumes of resin. In order to keep the temperature of the block to a minimum small volumes should be used. The volume used should be enough to ensure good infiltration and to provide enough resin to surround the tissue for subsequent handling and sectioning.

4. Temperature of polymerisation

UNICRYL may be polymerised by heat at 55-60C for 2 days or with UV light at -10 to 20C for two days depending upon the requirements of the tissue antigens.

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UNICRYL will not polymerise satisfactorily below -10C and is not designed to do so. The main reason for using UV light to polymerise the resin at low temperatures (ie from -10C to +20C) is to avoid raising the temperature of the tissue above 37C, the temperature at which most tissue antigens are stable. It is therefore recommended that resin blocks are polymerised at 55C thermally if the tissue antigens are not temperature sensitive. If tissue antigens are temperature sensitive then UV polymerisation may be used at 4C to 20C to prevent the tissue rising above 37C. Polymerisation at 4C is the most common arrangement. If polymerisation at 20C is chosen then care must be taken to avoid raising the block temperature from exothermic reactions. Move the tissue block further away from the light source to control the temperature and to achieve an even polymerisation in 2-3 days.

In some cases, however, it is recommended that tissues be also dehydrated in alcohol at low temperatures by the progressive lowering of temperature method (PLT). This is to dehydrate tissues at low temperatures in order to avoid extracting alcohol soluble components which would otherwise be lost at room temperature. For the majority of tissues this is not an important factor. Electron microscopy using epoxy resins demonstrates, however, that relatively few tissues are affected by alcohol dehydration.

Even if tissues are dehydrated and infiltrated with resin at low temperatures (i.e. down to-50C or so) it is **not necessary** to polymerise the tissue at very low temperatures (i.e. below -10C) once it is infiltrated with UNICRYL. Antigens in tissues are perfectly stable at temperatures up to 37C (as they are in the living tissue) and many will happily remain stable at higher temperatures. It is necessary only to prevent the resin from rising to excessively high temperatures during the exothermic reaction of polymerisation. This may be done, if it is likely to be a problem to rise above 37C, by using UV polymerisation at 4C. There is very little reason for polymerising at -10C or lower except in tissues where there are significant concentrations of components which may be soluble in resin at higher temperatures. Polymerisation must be performed in one continuous operation and at one chosen temperature. It is reversible while partly polymerised since the partly formed polymer can be solubilised in unreacted monomer.

5. Polymerisation chamber

A simple UV polymerisation chamber is described in the UNICRYL technical Instruction booklet. The light intensity and specimen-to-light distance is also recommended. It **cannot** be assumed that UV lighting conditions used for polymerising other types of resin will be suitable for polymerising UNICRYL. These other types of polymerisation chambers have been designed for different types of resin. Most difficulties arise due to insufficient UV irradiation intensity at incorrect temperatures. Following the instructions in the booklet will give good polymerisation.

6. Trouble shooting – extra observations

Most methods for handling the UNICRYL resin are described above. Below are some other observations that sometimes occur.

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Q1. The sections do not float on a water bath but appear to hygroscopic. The block face appears to wet and cause the water to travel onto the back of the knife. the blocks are soft and rubbery.

The block is not fully polymerised. if polymerisation is not complete then the resin remains more hydrophilic and attracts water. In addition tissue components are not fully retained and may fall out of the tissue block while floating on the water. This would be observed as a lack of staining or only partial staining. It is necessary to fully polymerise by continuous radiation with UV light with the control intensity such that full polymerisation occurs in 2-3 days at 4C.

Q2. The resin disappears during polymerisation in flat embedding moulds. The volume of resin shrinks during polymerisation.

See Note 1 above. There may be evaporation from or penetration of the resin into the mould material.

Q3. The blocks appear bubbly and cracked following polymerisation.

See (3) above. polymerisation is too rapid. reduce the light intensity to control block temperature.

Q4. Some tissue areas cut and stain well but others do not. Some blocks are polymerised while others are not.

Polymerisation is uneven. See (3) above. most probably the outer part of the block is polymerised but the centre is not. This may be due to the size of the resin block. It may also be due to insufficient UV light reaching the centre of the block through shielding of the block by the capsule or holder. Ensure that the whole of the resin block is freely irradiated by the UV light through the thinnest part of the capsule and is not shielded from light.

Q5. Bubbles appear in the block and ultrastructure is disrupted.

Tissue may have been insufficiently dehydrated. There may be microscopic traces of water or alcohol present. Prolong the dehydration schedule and allow adequate infiltration with resin.

Q6. Immunogold labelling of tissue proteins is unusually low.

a) Tissue antigens may have been damaged through excessive temperatures.
b) Polymerisation may be incomplete leading to increased hydrophilicity of the block and poor retention of the tissue. Tissue antigens may then be partly lost or denatured while floating sections on water or buffers. (See Q1 above).

Q7. Does UNICRYL have natural fluorescence?

The fluorescing properties of UNICRYL are very low. The resin may therefore be used for demonstrating fluorescing antibodies in tissue sections.

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Q8. Can UNICRYL be polymerised quickly?

Small blocks of UNICRYL can be rapidly polymerised either thermally or by UV light. The general rule is 4 hours for each linear 1mm of resin. To shorten the polymerisation time, **do not** raise the temperature or use a higher UV radiation intensity. This will only cause brittle blocks.

Q9. Cells embedded in UNICRYL shrink away from the resin in sections in the electron microscope.

This may happen because of differences in electron density between the cells and the surrounding resin. Cells may shrink away from the resin during exposure to the electron beam. This would be less likely in a tissue matrix where cells are surrounded by other proteins. The problem is less severe in epoxy resins which have a high degree of cross linking. Isolated cells may therefore withstand such irradiation damage if they are first surrounded by a material such as gelatin or albumin during the processing and embedment. Carbon coating sections or supporting them on plastic/carbon membranes may reduce this possibility.

Q10. Ripples appear in sections.

UNICRYL will absorb a small volume of water, up to 10%. If the tissue is not fully polymerised it may be that the tissue has not been fully dehydrated in alcohol. This would be seen by the presence of small ripples in various places in the sections together with some small bubbles. This may be more likely in plant tissues where the water content is higher. Even when using 100% alcohol there is still some uptake of water by the alcohol which may prevent the full dehydration of the tissue. Wherever possible new, dry alcohol should be used and a lengthy infiltration with resin should take place before polymerisation.

Q11. Can UNICRYL be used for calcified and hard tissues?

UNICRYL may be used like any other resin to embed hard tissues. The cutting properties of UNICRYL allow good sections to be obtained if the resin is fully polymerised. If polymerisation is incomplete the hard tissues will fragment while sectioning.

Q12. Will ultrathin UNICRYL sections adhere to plastic membranes?

Sections of UNICRYL will attach to most plastic membranes used in EM studies as well as to bare metal grids. They attach to membranes by hydrophobic attraction even though the resin is largely hydrophilic. Excessive use of detergents such as Tween 20 may remove the sections during incubations. When mounted on plastic membranes the sections should be first flattened to maximise the contact. Warming the sections first on a water surface may help to stretch them.

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Q13. Does alcohol affect polymerised UNICRYL?
Yes, alcohol does slightly affect polymerised UNICRYL. Blocks may interact on the surface and feel greasy if immersed in alcohol and sections may disintegrate if alcohol is present in the water bath

<u>Hazard Information</u>

Product:	UNICRYL
Product Code:	BA250
UN Hazard Class	3
UN Number	1993
Chemical Abstract Service Number	n/a

Physical Data

Description:	Pale yellow liquid, pungent odour
Meltin Point:	n/a
Boiling Point:	n/a
Specific Gravity:	1.024
Solubility in water:	Slightly miscible
Fire and Explosive Hazard:	Flammable
Flash point:	n/a
Explosion limits:	**(lower)** unknown **(upper)** unknown
Auto-Ignition temperature:	unknown
Firefighting Measures:	Dry powder, vapourising liquid, carbon dioxide.

Health Hazards
The toxicological properties of this product have not been thoroughly investigated. Exercise appropriate precautions to prevent any opportunities for direct contact with skin or the eyes, and to prevent inhalation. Should be treated as a suspect carcinogen.
Toxity data: Unknown. May cause skin irritation or sensitisation.
Carcinogenicity: Some components have been known to cause cancer in laboratory animals.
Mutagenicity/teratogenicity: May cause adverse mutagenic or teratogenic effects.
Exposure limits OES: Not assigned
MEL: Not assigned

First Aid

Eyes:	Wash with copious amounts of water for at least 15 minutes
Lungs:	Remove from exposure.
Skin:	Wash thoroughly with soap and water.
Mouth:	Wash thoroughly with water.

In all cases OBTAIN MEDICAL ATTENTION.

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Reactive Hazards

Stability: May polymerise on exposure to heat or light.
Reaction with water: None.

Other Known Hazards

Avoid contact with: Water (No); Acids (No); Bases (No); Oxidisers (Yes); Combustibles (No).

Spillage Disposal

Precautions: Wear suitable gloves, laboratory coat, boots and eye protection. In confined areas, or if a large amount is to be dealt with, wear appropriate NIOSH approved respirator.

Procedure: Absorb material onto vermiculite or other suitable absorbent and place in an impervious container. Wash affected area with detergent and water. Dispose of by incineration, or contract with licensed waste disposal company. Alternatively polymerise with UV light and dispose of with normal chemical waste.

Protective Measures: (as appropriate to quantity handled)

Respirator: If local ventilation controls are inadequate use appropriate NIOSH approved respirator.
Ventilation: Use in an efficient fume hood.
Gloves: Wear an acrylate resistant brand, such as those supplied.
Eye Protection: Always wear safety glasses.
Other:

Storage and Handling

Special requirements: Store at 4°C or -20°C in the dark in the container provided.

Additional Comments:

hazardous contents >1%:

Monomeric(meth-)acrylate esters 94%
Styrene monomer 4%

See additional notes overleaf about these materials.

THE INFORMATION ON THIS LEAFLET IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT TO BE ALL INCLUSIVE AND SHALL ONLY BE USED AS A GUIDE. BBI Solutions SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT.

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Component – Styrene (Monomer)

Health Hazards

Harmful by ingestion and inhalation. Irritating to skin, eyes and respiratory system. Vapour is narcotic in high concentrations. Prolonged exposure to high concentrations may produce systematic effects.

Toxicity data:	LD50 5000mg/kg oral, rat.
Carcinogenicity:	Has been found to cause cancer in laboratory animals.
Mutagenicity/Teratogenicity:	Evidence of reproductive effects.
Exposure limits:	**OES:** n/a **MEL:** 420 mg/m3 (long term, 8 hour TWA)

Reactive Hazards

Stability:	Polymerises on exposure to light and heat.
Reaction with Water:	None.
Other Known Hazards	Sources of free radicals can cause runaway polymerisation. Reacts with oxidisers.

Additional Comments

Because of the hazardous nature of styrene, and mixtures containing it, the need for good lab hygiene can only be stressed once more. The safety procedures detailed overleaf should be strictly adhered to.

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Component – Monomeric (meth-) acrylate esters

Health Hazards

Harmful by ingestion and inhalation. Irritating to skin, eyes and respiratory system. May cause sensitisation if contact is prolonged.

Toxicity data:	Unknown.
Carcinogenicity:	No evidence of carcinogenic properties.
Mutagenicity/Teratogenicity:	No evidence of mutagenic or teratogenic effects.
Exposure limits:	**OES:** n/a **MEL:** n/a

Reactive Hazards

Stability:	Stable.
Reaction with Water:	None.

Other Known Hazards

Additional Comments

Methacrylate mixtures should always be handled in a fume cupboard taking the precautions advised overleaf to minimise any possibility of contact with eyes or skin, and to reduce the chance of inhalation of the vapour.

Risk Codes Associated with this product

R8/10/17/20/36/37/38/41/43.
S9/16/23/24/25/26/28/33/36/37/39.