

Agar Low Viscosity Resin (LV) Kit

AGR1078



Instructions for use

Agar low viscosity resin is our replacement for SPURR, a widely used embedding resin for electron microscopy. ERL 4206, a major component of Spurr is no longer available. The low viscosity resin offers all the advantages of Spurr, i.e. rapid penetration, good contrast, easy sectioning, stability under the electron beam, satisfactory staining of most thick sections for light microscopy and good staining of thin sections for electron microscopy. It has none of the known carcinogenic effects of Spurr, but as with all resins, proper handling precautions such as the use of gloves and a fume hood or a well-ventilated area should be observed.

Recommended Procedures

Fixation of tissues can be in a wide range of fixatives including the commonly used process of glutaraldehyde followed by osmium tetroxide.

Dehydration schedules will depend largely on the type and size of specimen that is being processed but a typical one is as follows:

70%	Ethanol for 10 minutes
100%	Ethanol for 10 minutes
100%	Ethanol for 15 minutes
100%	Propylene Oxide (or acetone) for 15 minutes 100%
	Propylene Oxide (or acetone) for 15 minutes
50:50	Embedding medium/propylene oxide for 30 minutes
100%	Embedding medium for 30 minutes to 2 hours at room temperature.

For many specimens, the propylene oxide stage may be omitted although infiltration using ethanol/resin mixtures instead of propylene oxide/resin mixtures takes longer. The ethanol must be totally dry. Traces of propylene oxide are able to cross-link into the final resin block (at least in moderation). Traces of ethanol are more likely to remain and can more readily affect the consistency of the final block.

Mixing Instructions

A suitable mixture for a medium hardness block is as follows:

LV Resin	48.0g
VH1 Hardener	16.0g
VH2 Hardener	36.0g
LV Accelerator	2.5g

Harder Blocks may be obtained by replacing part of the VH1 with VH2, and softer blocks by replacing part of the VH2 with VH1

Other combinations might be as follows:

	Soft	Hard
LV Resin	48g	48g
VH1 Hardener	26g	-
VH2 Hardener	26g	52g
Accelerator	2.5ml	2.5ml

Before measuring and mixing, the hardeners may be warmed to 60°C to reduce their viscosity if required. Thorough mixing is imperative to be able to achieve uniform blocks but try to avoid an excessive incorporation of air bubbles.

It is recommended that freshly prepared embedding medium is always used but pre-mixed embedding resin can be stored at –20°C for many months although it will thicken slowly even at this low temperature. If you choose to store the mixture at low temperature, you should warm it thoroughly before use to avoid water vapour condensing into the resin. This could affect curing and the final hardness of the block.

The above schedule may be shortened for small or thin specimens or with extensive experience of your material.

Infiltration

It is recommended that for all of the infiltration steps a specimen rotator be used. Drain the tissue of most of the propylene oxide, leaving a little so it does not dry out. Replace the solvent with a 1:1 solution of propylene oxide/embedding medium and allow it to stand for at least 1 hour at room temperature. A second change of 2:1 embedding medium to propylene oxide at room temperature overnight is recommended for dense tissues.

Embedding

This may be done in many of the capsules and embedding moulds shown in the Agar Catalogue. Transfer each sample to a dry capsule or mould and fill with embedding medium. Cure the medium in an oven at 60°C for 16-24 hours. Curing at 70°C may also be used where faster results are needed. Blocks can be sectioned after they have returned to room temperature.

Storage

Components can be stored at room temperature in stoppered bottles. The shelf life of unopened resin and hardener is several years. Partially used components may be used as long as they are well capped, but there is always the possibility of atmospheric water ingress that will affect curing and block hardness.

Accelerator is best date-marked on arrival and used within 12 months. Symptoms of aging accelerator are various but may be slow, uneven curing, brittleness, colour change, difficult sectioning and poor resistance to the electron beam.