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Glauert Araldite Embedding Medium

ARALDITE:

The first epoxy resin embedding medium for Electron Microscopy was developed by Audrey Glauert and her colleagues[1,2] in the 1950's and was based on the Araldites, the epoxy resins manufactured by Ciba-Geigy. The same embedding medium is still in use today, with only minor modifications from the original formulation, and it has proved to be excellent for the examination of all types of biological specimen. Araldite blocks have ideal mechanical properties for the cutting of very thin sections of high quality and the sections are more stable during irradiation in the electron microscope than sections of any other embedding medium developed for electron microscopy.

GLAUERT ARALDITE EMBEDDING MEDIUM:

The embedding medium is very simple since it contains only three components: an epoxy resin, an anhydride hardener, and an amine accelerator. Only the amount of the accelerator needs to be measured accurately.

The epoxy resin is either Araldite CY212 (which is the same as the Araldite M of the original formulation) or its US equivalent Araldite 502 (which was introduced by Finck in 1950)5.They are both aromatic epoxy resins and contain dibutyl phthalate (DBP) as a plasticizer. There is 24% dibutyl phthalate by weight in Araldite M (CY212) and 17% in Araldite 502.

The Hardener is dodecenylsuccinic anhydride (DDSA).

The accelerator is benzyldimethylamine (BDMA) and replaces the DMP-30 of the original formulation. BDMA should always be used in epoxy resin embedding media in preference to DMP-30. BDMA has a lower viscosity, penetrates tissue better and has a longer shelf life (see Glauert 1987)[3].

STANDARD ARALDITE EMBEDDING MEDIUM:

- AralditeM (CY212) orAraldite502 ..... 20.0mL
- Hardener,DDSA 22.0mL
- Accelerator,BDMA 1.1mL

The anhydride: Oepoxide ratio varies from 0.80 to 0.85, as the weight per epoxide of the Araldite varies within the limits stated by Ciba-Geigy. Blocks of Araldite 502 are slightly harder than blocks of Araldite M (CY212), but this is easily adjusted (if necessary) by the addition of 0.6ml DBP to the standard formulation for Araldite 502, so that the content of DBP in now 24%.

For a softer bock (which is rarely required), add a small amount of a reactive flexibilizer (see Glauert 1974),[4] such as DER 736, a diglycidyl ether of polypropylene glycol. Alternatively, add some additional dibutyl phthalate. For harder blocks, replace 1.00 mL of DDSA with 0.5 mL of methyl nadic anhydride (MNA). This will maintain the anhydride; epoxide ratio at 0.80 to 0.85.

The epoxy resins and hardeners, DDSA and MNA, can be stored indefinitely at room temperature. The accelerator, BDMA, must be kept dry. Ensure that the bottle is firmly stoppered and, if possible, place the bottle in a desiccator. Do not refrigerate any of the components of the embedding medium.

PREPARATION OF ARALDITE EMBEDDING MEDIA:

Complete mixing of the embedding medium components is easy if the resin and hardener are warmed to reduce their viscosity. This is done by placing the containers of Araldite and DDSA, together with a graduated cylinder and a conical flask, in an oven at 60°C.

To prepare the embedding medium, pour the required quantities of warm Araldite M (CY212 or 502) and DDSA into the warm graduated cylinder and then immediately pour the mixture into the warm conical flask. Rotate the flask by hand for a few minutes until mixing is complete. Then add 1.1 ml of the accelerator BDMA, measured accurately with a pipette, for every 42ml of the Araldite/DDSA mixture and continue shaking the flask by hand for a further minute or two. The embedding medium is now ready for use.

The graduated cylinder (and later the conical flask) should be drained immediately after use by inverting them over a disposable container. They can then be used again and no washing up is required.

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TECHNICAL DATA SHEET 491

EMBEDDING SPECIMENS IN ARALDITE:

Specimens, which have been fixed and dehydrated by standard techniques,[4] are placed in small vials and are infiltrated with Araldite embedding medium as follows:

100% ethanol or acetone/
propyleneoxide(1/1) ..... 10min.

Propyleneoxide..... 10min.

Propylene Oxide/
Araldite embedding medium (1/1) . . . 1 hr (or longer)

Araldite embedding medium ..... 2 hr (or longer)

The standard infiltration schedule is suitable for the majority of specimens, but modifications may be required for large specimens or dense tissues to ensure adequate infiltration. Similarly, the times can be reduced for very small specimens and pellets.

Propylene oxide is volatile and flammable. Infiltration should be carried out in a fume hood and the propylene oxide should be disposed of in accordance with local environmental laws.

The same batch of Araldite embedding medium can be used throughout, but, when necessary fresh medium is easily prepared in a short period of time. The Araldite embedding medium should be warmed to 60°C, before each change, to reduce its viscosity. Infiltration should also be assisted by placing the vials in a specimen rotator. Any excess Araldite media should be put in a plastic container and hardened before disposal.

The specimens are now transferred to capsules or flat embedding molds, filled with Araldite embedding medium, and the Araldite is hardened by heating at 60°C, in an embedding oven for 48 hours. Blocks can be sectioned after curing for only 16-24 hours, but sectioning is improved if a longer time is used. The 48-hour time is not critical and the blocks will not change significantly if they are left for a longer time. The temperature of curing should not be increased above 60°C, since this may result in a brittle block.

Araldite blocks section easily on the ultramicrotome and the sections are stable in the electron microscope, so that they can be mounted on uncoated grids.

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3. Glauert, A.M., “Accelerators for Epoxy Resins”, Proc. Roy. Microsc. Soc., **22**, 264 (1987).
4. Glauert, A.M. “Fixation, Dehydration and Embedding of Biological Specimens, Practical Methods in Electron Microscopy, **3**, A.M.Glauert, ed. (North-Holland/Elsevier, Amsterdam), (1974).
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ORDERING INFORMATION:

Cat. #	Description	Size
00552	Araldite 502	500g
00563	DDSA	450g
		4 x 450g
00141	BDMA	100g
		500g
00886	Methyl Nadic Anhydride(MNA)	500g
02923	DER 736	450g
00434	Dibutyl phthalate	450g

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