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AGAR GMA (HEMA) KIT

AGR1036

Each Kit Compromises of:

5 x 100g	2-Hydroxyethyl methacrylate
1 x 100g	2-Butoxyethanol
1 x 100g	Carbowax 400
1 x 25g	Benzoyl peroxide, damped
1 x 25g	Dimethylaniline

Instructions for use

Processing schedule for 2-hydroxyethyl methacrylate (HEMA)/GMA

Following fixation pass through ascending grades of ethanol (50%, 70%, 80% and 90%) to three changes of absolute ethanol. The time of exposure at each stage should be 30-45 minutes.

Following dehydration give three changes of 45 minutes each in solution A (see below). Infiltration is complete when the tissue becomes translucent. Occasionally tissues such as lung may benefit from infiltration under slight negative pressure.

Embed in 50 parts of solution A to one part of solution B (see below). A suitable embedding mould is a BEEM capsule. The conical tip is cut off just before the mould tapers and a cap from another mould placed over the cut end, which can now be used as the mould base.

Polymerisation occurs at room temperature and should be carried out under anaerobic conditions as contact with the atmosphere will inhibit polymerisation. BEEM capsules should be firmly sealed or, in the case of the Sorvall mould system, all areas where atmospheric contact is likely, sealed with paraffin wax. Polymerisation is usually complete 4-6 hours but can be conveniently left overnight. Should the blocks be sticky or rubbery the next day, rinse in ethanol and give a short exposure in a 60 C incubator which will harden them completely. Very occasionally excessively hard or brittle blocks are produced. Subsequent, sectioning of these blocks may be facilitated by treating them at 37 C for 4-16 hours in a sealed container containing a wad of cotton wool soaked in 70% ethanol. Polymerised blocks may be clamped directly in a microtome or stuck to a suitable base using cyanoacrylate adhesive.

Processing can also be completed without alcohol as HEMA is a water-soluble resin. In this case give changes of 30-45 minutes in 25% solution A in distilled water, 50% solution A in distilled water and 75% solution A in distilled water then three changes of 45-60 minutes each in solution A and proceed as in the previous technique.

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Solution A

2-Hydroxyethyl methacrylate	80ml
2-Butoxyethanol	15 ml
Benzoyl peroxide	0.3g

The ratio may be varied from 7:3 to 3:7 depending on block hardness required. Dissolve using a magnetic stirrer, but no heat

Solution B

Polyethylene glycol 400	10ml
N, N-dimethylaniline	1ml

Both solutions should be stored at +4°C but must be allowed to attain room temperature before use. At +4°C this solution will keep approximately one month.

An inhibitor (often methoxy ethyl hydroquinone) is present in 2-hydrosyethyl methacrylate, the proportion of which may vary with the manufacturer. This will affect the amount of benzoyl peroxide needed in solution A, and it may be wise to alter the percentage use until a polymerisation time of about 4-6 hours is achieved. Excess humidity in the laboratory will also adversely affect polymerisation.

CAUTION: Benzoyl peroxide is explosive when dry. Beware spent filter paper etc.