

Technical Data Sheet

Technovit H8100 for Immunohistochemistry

#14654

Glycol Methacrylate Embedding For Immunohistochemistry

Embedding in plastic provides many advantages to the histotechnologist. Thinner sections can be made providing improved detail. Better support is given to cellular components, offering improved morphology. Short, straight forward protocols are available giving minimum processing times. In addition, improved chemistry and protocols are now available that are more antigen-friendly.

This protocol will help get you started with embedding your soft biological samples in Technovit 8100, a GMA kit sold by Electron Microscopy Sciences. Note the areas in which it deviates from the guidelines for embedding in GMA for morphology only.

Procedure

1. Use gentle agitation during fixation, washing, dehydration and infiltration for best results.
2. Fixation of the biological samples can be done in any way appropriate for the antigen you are seeking. Cold acetone, periodate/lysine/paraformaldehyde, and buffered paraformaldehyde solutions have all been used successfully. Try to keep the sample size small using 10mmx10mmx2mm as a maximum. Fixation should be done for several hours at 4°C.
3. Wash out the fixative with 6% sucrose solution at 4°C overnight.
4. Dehydration for GMA embedments does not have to be complete because of GMA's miscibility in water. Use acetone for one hour at 4°C changing the acetone at the beginning until it remains clear.
Make up your infiltration solution from:
100ml of Technovit 8100 resin, i.e. 2-hydroxyethyl Methacrylate (GMA)
0.6gm of Hardener I (benzoyl peroxide)
5. Mix using a magnetic stirrer until the benzoyl peroxide is complete dissolved. Store at 4°C in a dark bottle for up to one month.
6. Infiltrate for six to ten hours at 4°C using mild agitation. Vacuum will make infiltration more complete and larger samples should have more infiltration steps over a longer period of time.
Make up your embedding solution from:
30ml of infiltrating solution
1ml of Hardener II
7. Mix for one minute using a magnetic stirrer. Use the solution within 10 minutes, before polymerization occurs.
8. Cover your samples in the embedding molds with this solution. Molds of polyethylene such as the JB-4 mold or Peel-away molds are most often used. Block holder/adaptor in the recess on the mold and add mounting plastic.