System for the embedding and cutting of histochemical specimens

GUS staining

For GUS staining, tissues were prefixed in ice-cold 90% acetone for 20 min, rinsed with cold water, immersed with staining solution (50 mM sodium phosphate buffer, pH 7.0, 0.1% Triton X-100, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, and 1 mM X-Gluc), and incubated at 37°C for 2 to 5 h for roots and 20 h for rosette leaves and siliques. For higher integrity of the DIC imaging, destaining time was limited before fixation; therefore, very light staining does not indicate signal. The stained samples were fixed in 50% ethanol, 5% acetic acid, and 3.7% formaldehyde, dehydrated through an ethanol series

Embedded in Technovit 7100

・Pre-infiltration

Mixing ration : equal parts of 96% or absolute ethanol and base liquid Technovit 7100 (Heraeus Kulzer). Specimens remain in the solution for 1-2 hours, according to size.

・Infiltration

1 g hardener Ⅰ is solved in 100 ml base liquid(approx. 5 min). The specimens are infiltrated in a sufficient amount of preparation solution for 1-12 h, depending on specimen thickness and type of tissue.

・Polymerization

1 ml hardener Ⅱ is added with the help of a pipette and stirred into 15 ml of preparation solution. Please use customary dosing devices. 1-3 ml of the solution are poured into Histoform S or Q, then the infiltrated specimens are placed in the form and positioned as required. Time of workability (pot life) at room temperature (23℃) is approx. 5-7 min. At room temperature (23℃) the specimens will cure within approx. 2h.

Mounting

The cured specimen in the embedding mould Histoform S or Histoform Q is mounted with the help of Technovit 3040.

-Place the Histobloc in the recess of the embedding mould Histoform S or Q.

-Mix Technovit 3040 in a volume ratio of 2 parts powder to 1 part liquid to obtain a viscous liquid.

-Pour Technovit 3040 into the recess at the back of the Histobloc to a level of about 2 mm above the base of the Histobloc.

-After about 10 min. The Histobloc together with the fixed specimen can be removed from the Histoform.

Cutting with Histoknife

-Loosen the clamping screws of the knifeholder
-Place the Histoknife in the knifeholder of the microtome (LEICA RM2155)
-Clamp the Histoknife into the knifeholder by tightening the screws
-Place the holder with the blade in the microtome and fasten down
-Adjust the cutting angle
-Cut

Tissue sections (10 µm thick) were mounted on slides and examined with a light microscope (Zeiss).

Reference

Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, Lee OR, Richards EL, Murphy AS, Sato F, Yazaki K.(2005)
PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *The Plant Cell*, 17, 2922-2939