

Fluorescent Immunolocalization of Arabinogalactan Proteins and Pectins in the Cell Wall of Plant Tissues

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Materials

Name	Company	Catalog Number	Comments
25% (w/v) Gluteraldehyde	Agar Scientific	AGR1010	aq. Solution, methanol free
8 wells Glass reaction slides	Marinfeld	MARI1216750	other brands may be used
Acetic acid	Sigma-Aldrich	A6283	
Anti-Rat IgG (wole molecule)-FITC antibody produced in GOAT	Sigma-Aldrich	F6258	
cover slips, 24 mm x 50 mm	Marinfeld	MARI0100222	The cover slip should cover all the wells. Other brands may be used
ddH ₂ O	na	na	
Absolute ethanol	na	na	
Fluorescent brightner 28	Sigma-Aldrich	F-6259	
Gelatin capsules	Agar scientific	AGG29211	The capsule size should fit the size of the sample.
Glass vials	na	na	Any simple unexpensive glass vials that can be sealed, may be used. The vials may be cleaned with 96% ethanol after use to remove LR-White residue and reused.
LR-white medium grade, embedding resin	Agar Scientific	AGR1281	LR-White comes in several forms; the medium grade provides an adequate cutting support for most tissues, while for harder tissues a harder grade of LR-white may be recommendable. If possible use a resin already mixed with the polimeration activator (benzoyl peroxide), if not please follow the instructions of the supplier to prepare the resin.
Non fat dry milk	Nestlé	na	any non fat dry milk is adequate
Oven	na	na	generic laboratory oven
Petri dish, 10 cm x 10 cm square	na	na	
PIPES	Sigma Aldrich	P1851	

Rat generated Monoclonal Anti-Body	Plant probes	na	Several antibodies that recognize cell wall components are available at both the Complex Carbohydrate research center (CCRC, Georgia University USA) and Plant Probes (Paul Knox Cell Wall Lab, at Leeds University UK). A short list of some commonly used MABS and where they can be purchased is presented in Supplemental Table 1
Razor blades	na	na	regular razor blades
SDS	Sigma-Aldrich	L6026	
Toluidine Blue-O	Agar Scientific	AGR1727	
Tween 20	Sigma-Aldrich	P9416	
Ultramicrotome	Leica Microsystems	UC7	
Upright epifluorescence microscope with UV and FITC fluorescence filters	Leica Microsystems	DMLb	
Vacuum chamber	na	na	
Vacuum pump	na	na	
Vectashield	vecta Labs	T-1000	Other anti-fade may be used. Please do check for compatibility with FITC and the Fluorescent brightner 28. (Note: for a non-commercial alternative, (see Jonhson et al 1982 ¹⁸) An anti-fade medium can be made by mixing 25 mg/mL of 1,4-Diazobicyclo-(2,2,2)octane (DABCO) in 9:1 (v/v) glycerol to 1xPBS. Adjust pH to 8.6 with diluted HCl.)