

Materials List for:

Whole-mount Clearing and Staining of *Arabidopsis* Flower Organs and Siliques

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Materials

Name	Company	Catalog Number	Comments
Reagents and Materials			
Ethanol	Scharlau	ET00102500	
Acetic Acid	Applchem	A3686,2500	100% Molecular biology grade
Glacial Acetic Acid	Sigma-Aldrich	320099	Molecular Biology Grade
Methanol	Scharlau	ME03062500	
Formaldehyde Solution	Sigma-Aldrich	F1635	
Propionic acid	Sigma-Aldrich	81910-250 ml	
Chloral hydrate	Sigma-Aldrich	15307	
Glycerol	Roth	3783.1	
Gum arabic	Fluka	51198	
Lactic acid	Fluka	69773	
Phenol	Sigma-Aldrich	77607-250ML	We used liquid phenol (use the density to find the required volume for your solution)
Clove oil	Sigma-Aldrich	C8392-100ML	
Xylene	Roth	4436.1	
Iodine	Fluka	57665	
Potassium iodide	Merck	5043	
Malachite Green	Fluka	63160	
Fuchsin acid	Fluka	84600	
Orange G	Sigma	7252	
Sodium Dodecyl Sulfate	Sigma-Aldrich	L3771	Molecular Biology Grade
Sodium hydroxide	Sigma-Aldrich	71690	
Sodium di-Hydrogen Phosphate	Applchem	A1047,1000	
Sodium phosphate dibasic	Sigma-Aldrich	S9763-1KG	
Potassium phosphate	Sigma-Aldrich	04347	
EDTA	Applchem	A2937,1000	
Calcofluor	Sigma	F6259	Fluorescent brightener 28
Auramine	Chroma	10120	
DAPI	Sigma	D9542	toxic
Triton-X-100	Sigma	T8787	
Aniline blue	Merck	1275	
MS medium	Carolina	19-57030	
Nutrient-rich substrate	Einheitserde	ED73	
Watch maker's glass			No specific brand

15 ml falcon centrifuge tubes	VWR	62406-200	
Dumond Forceps	Actimed	0208-5SPSF-PS	
Forceps	DUMONT BIOLOGY	0108-5	
Syringe	BD	BD Plastipak 300013	1 ml
Preparation needle	BD	BD Microlance 304000	
Microscope slides	Thermo Scientific	10143562CE	cut edges
Coverslips	Thermo Scientific	DV40008	
Humid box			A plastic box with damp paper towel and slide supports inside
Name	Company	Catalog Number	Comments
Solutions			
Fixatives			
Carnoy's (Farmer's) fixative			Absolute ethanol : glacial acetic acid, 3:1 (ml:ml)
Methanol/acetic acid fixative			50 % (v/v) methanol, 10 % (v/v) glacial acetic acid in deionized water
FPA50 fixative			Formalin, propionic acid, 50% ethanol; 5:5:90 (ml:ml:ml)
Clearing solutions			
Chloral hydrate/glycerol			Chloral hydrate : glycerol : water, 8:1:2 (g:ml:ml). Can be used for all flower developmental stages and for silique development with DIC microscopy. The best fixative is the formaline based FPA50
Modified Hoyer			Gum arabic 7.5 g, chloral hydrate 100 g, glycerol 5 ml , water 30 ml. Can be used for all flower developmental stages and for silique development with DIC microscopy. The best fixative is the formaline based FPA50
Herr's 4½ clearing fluid			Lactic acid, chloral hydrate, phenol crystals, clove oil, xylene; 2:2:2:2:1, by weight. Can be used for all flower developmental stages (especially for stamen development) and for silique development with DIC microscopy. The best fixative is the formaline based FPA50
SDS/NaOH solution			Mix-dilute the the SDS and the NaOH stock solution to 1% SDS / 0.2 N NaOH (10x dilution). For all stages of flower and silique developmental stages. The best fixative is the methano/acetic acid fixative; the other two fixatives can also be used. Can be combined with calcofluor, auramine, DAPI, and aniline blue staining solution.
SDS stock solution			10 % (w/v) sodium dodecyl sulphate. Dissolve 10 g sodium dodecyl sulphate in 80 ml deionized water and make up to 100 ml with deionized water.
NaOH stock solution			2 N NaOH solution: dissolve 4 g of NaOH in 40 ml of deionized

			water and make up to 100 ml with deionized water
Combined clearing and staining solutions			
Herr's IKI-4½			To a standard 4½ (9 g in total) add: 100 mg iodine, 500 mg potassium iodide. This clearing solution can be used for all flower developmental stages and for silique development, either for increasing contrast or for characterizing starch dynamics. Use FPA50 for structural analysis and Carnoy's fixative for quantitative starch analysis.
Alexander staining			Ethanol 95% 10ml, malachite green (1% in 95% EtOH) 1 ml, fuchsin acid (1% in ddH2O) 5ml, orange G (1% in ddH2O) 0.5ml, phenol 5g, chloral hydrate 5g, glacial acetic acid 2ml, glycerol 25ml . This clearing/staining alone or in combination with Herr's 4½ solution can be used to evaluate pollen abortion in flowers with mature and tricellular pollen grains. It's used on freshly harved non-fixed material.
Staining solutions			
Calcofluor solution			Calcofluor 0.007% in water (g:ml). Originally used as an optical brightner. Can be used for staining cellulose, carboxylated polysaccharides and callose in cell walls. Frequently used to stain the intine of the pollen grain. All three fixatives can be used with this solution.
Auramine solution			Auramine 0.01% in water (g:ml). This lipophilic fluroscent dye can be used for staining cuticles, cutin, and exine among others. All three fixatives can be used with this solution
Calcofluor-Auramine mixture			Auramine solution : Calcofluor solution, 3:1. Can be used for a combined staining by both solutions. Other proportions can be assayed maintaining a smaller proportion of calcofluor with respect to auramine.
DAPI solution			DAPI 0.4 ug/ml, 0.1 M sodium phosphate buffer (pH 7), 0.1% Triton-X-100, 1 mM EDTA. This solution can be used for staining chromosome spreads during male and female meiosis, and cell nuclei of any tissue. Frequently used for studying pollen grain development. Carnoy's and methanol/ acetic acid are the best fixatives for this solution. Formaldehyde-based fixatives such as FPA50 may interfere with the staining.

			Excitation in the UV and maximum emission around 461 nm.
Sodium phosphate buffer (0.1 M)			Proton receptor: 0.2 M Na ₂ HPO ₄ , proton donor: 0.2 M NaH ₂ PO ₄ , ratio proton donor / proton receptor: 1.364 (for a pH 7)
Aniline blue solution			0.1% (w/v) aniline blue, 108 mM K ₃ PO ₄ (pH 11), 2% glycerol. This solution can be used for staining callose and cellulose of many stages of development (e.g callose deposition in male and female terads, callose plugs in pollen tubes). Excitation in the UV and maximum emission around 455. It can also be excited at 514 nm with emission in the red for cell content staining.