

Materials List for:

Identification of Critical Conditions for Immunostaining in the Pea Aphid Embryos: Increasing Tissue Permeability and Decreasing Background Staining

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Materials

Name	Company	Catalog Number	Comments
30% hydrogen peroxidase (H ₂ O ₂)	Sigma-Aldrich	18304	For bleaching endogenous peroxidase of embryos.
4'6-diamidino-2-phenyl-indole dihydrochloride (DAPI)	Sigma-Aldrich	D9542	For labeling the double-strand DNA. TOXIC. Wear gloves, dispense into small aliquots and freeze to minimize exposure
4D9 anti-engrailed/invected mouse monoclonal antibody	Developmental studies hybridoma bank	AB_528224	For labeling the developing segments of embryo.
ApVas1 rabbit polyclonal antibody	Our laboratory	N/A	For labeling the aphid germline specific Vas1 protein in embryos. This antibody was made by our laboratory.
Austerlitz Insect pins	ENTOMORAVIA	N/A	For separating each egg chambers from an ovariole. Size 000, BLACK ENAMELLED.
Bovine serum albumin (BSA)	Sigma-Aldrich	9048-46-8	For blocking the non-specific binding of antibodies.
Camera connted to compound microscope	Canon	EOS 5D	For photographing of aphid embryos.
Colorimetric 8 cell tray	Kartell Labware	357	For developing the staining signal of aphid embryos. Diameter 95 x 57 mm, cell depth 2 mm.
Compound microscope with DIC optics	Leica Microsystems	DMR	For photographing of aphid embryo mounted on the slides.
DIG Wash and Block Buffer Set	Sigma-Aldrich (Roche)	11585762001	For blocking the non-specific binding of antibodies. We diluted the 10x Blocking solution into 1x as blocking reagent.
Forceps	Ideal-tek	N/A	For dissection of ovaries from aphids. Manufacturer part No 5: 5 SA.
Glass dropper	N/A	N/A	For transferring ovaries of aphids from the spot plate to tubes. 150 mm of total length and 5 3/4" of tip length.
Glycerol	Sigma-Aldrich	G5516	For clearing of aphid embryos and mounting.
Glycine	Bioshop	N/A	For blocking the enzyme activity of proteinase K (PK).

Goat anti-mouse IgG conjugated Alexa Fluor 488	Invitrogen	A11017	For detection of primary antibody from mouse.
Goat anti-rabbit IgG conjugated Alexa Fluor 633	Invitrogen	A21072	For detection of primary antibody from rabbit.
Intelli mixer	ELMI laboratory equipment	RM-2M	For improving the thoroughly reaction of reagents with ovaries.
Laser-scanning confocal microscopy	Leica Microsystems	SP5	For photographing of aphid embryo mounted on the slides with florecent tag.
methanol	Burdick and Jackson	AH2304	For bleaching endogenous peroxidase of embryos.
Microscope slides	Thermo scientific	10143560	For mounting of embryos. SUPERFROST ground edges, ca./ env. 76 x 26 mm.
Microscope Cover glasses	Marienfeld	0101030	For mounting embryos on the slides. Size 18 x 18 mm. Thickness No. 1 (0.13 to 0.16 mm)
Microscope Cover glasses	Marienfeld	0101050	For mounting embryos on the slides. Size 22 x 22 mm. Thickness No. 1 (0.13 to 0.16 mm)
mouse monoclonal anti-alphaTubulin antibody (DM1A)	Santa Cruz Biotechnology	sc-32293	For labeling the distribution of alpha-tubulin of cells.
Nail polish	N/A	N/A	For sealing the coverslips on the slides.
Normal goat serum (NGS)	Sigma-Aldrich	G9023	For blocking the non-specific binding of antibodies.
Paraformaldehyde (PFA)	VWR	MK262159	For fixation of aphid ovaries.
Phalloidin-TRITC	Sigma-Aldrich	P1951	For labeling the distrbution of F-actin on embryos.
Phosphate-buffered saline (PBS)	N/A	N/A	As isotonic solution for aphid ovaries.
Plastic dropper	N/A	N/A	For transferring ovaries of aphids from tube to cell tray. Size 3 ml.
Proteinase K	Merck	124678	For creating pores (punching) on the cell membrane and facilitating the access of antibodies.
Pyrex spot plate	N/A	N/A	For dissection and color reactions of aphid ovaries under microscopy. Each cavity with diameter 22 mm and depth 7 mm.
SIGMAFAST 3,3'-diaminobenzidine (DAB) tablets	Sigma-Aldrich	D4168	For developing of substrated signals. Also called DAB peroxidase substrate tablet set.
Stereo microscope	Leica Microsystems	EZ4	For dissection and observation of aphid ovaries.
Triton-X 100	Sigma-Aldrich	T8787	For creating pores (punching) on the cell membrane.
VECTASHIELD Elite ABC Kit (Rabbit IgG)	Vector laboratories	PK-6101	For enhancement of the signal. Including of biotinylated goat anti-rabbit IgG secondary antibody, A and B reagents.
VECTASHIELD mounting medium	Vector laboratories	H1000	For clearing of aphid embryos and mounting.