

Materials List for:

Dissection and Explant Culture of Murine Allantois for the *In Vitro* Analysis of Allantoic Attachment

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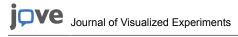
Materials

Name	Company	Catalog Number	Comments
C57BL/6J wildtype mice	The Jackson Laboratory	Stock No: 000664	
70 % (v/v) Ethanol	VWR International	930031006	
Standard pattern forceps	Fine Science Tools	11000-12	Forceps used to open the abdominal cavity.
Micro dissecting forceps	Fine Science Tools	11254-20	Dumont # 5 medical biology forcepts with fine, sharp tip.
Fine scissors	Fine Science Tools	14094-11	Straight blades.
Spring scissors	Fine Science Tools	15012-12	Noyes spring scissors with 14 mm blades for the dissection of uterus buds.
Microspoon	Carl Roth	AT18.1	5 mm Spoon diameter.
Microtiter plates	ibidi	81501	We use uncoated, sterile angiogenesis slides (internal volume 50 μL) with a hydrophobic surface that is not tissue-culture treated to reduce non-α4β1-mediated binding to plastic.
10 cm dish	Nunc	150350	Sterile tissue culture dishes.
3.5 cm dish	Nunc	150288	Sterile tissue culture dishes.
Large orifice pipette tips	Biozym	VT0140X	Low binding pipette tips, 200 μL.
α4β1 integrin	R&D Systems	6054-A4	Murine α4β1 integrin recombinantly expressed and purified from a Chinese hamster ovary cell line.
Dulbecco's Phosphate Buffered Saline (PBS)	Sigma Aldrich	D8537	Without Ca ²⁺ and Mg ²⁺ .
Dulbecco's Modified Eagle Medium (DMEM)	PAN Biotech	P04-03600	The formulation contains 4.5 g/L glucose, 110 mg/L sodium pyruvate and 3.7 g/L NaHCO ₃ but no L-glutamine, which is added separately.
Penicillin/Streptomycin	Gibco (ThermoFisher Scientific)	15140-122	100 x (10,000 U/mL Penicillin and 10,000 µg/mL streptomycin) stock solution. Prepare and store aliquots at -20 °C to avoid freeze/ thaw.
L-Glutamine	PAN Biotech	P04-80100	100 x (200 mM) Stock solution. Prepare and store aliquots at -20 °C to avoid freeze/thaw.
Fetal bovine serum	Biochrom	S 0115	We use heat-inactivated FBS (heated to 56 °C for 30 min with mixing to inactivate complement).

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Bovine serum albumin (BSA)	Sigma Aldrich	A7030	We use protease- and fatty acid- free albumin. Prepare a 0.1 % (w/ v) solution in DMEM. Sterile filter the solution through a disposable cell culture filter with a 0.22 µm pore size and low protein-binding membrane, and store aliquots at -20 °C.
<i>para</i> -Formaldehyde	Carl Roth	0335.2	To make a formaldehyde solution in PBS, weigh <i>para</i> -formaldehyde powder in a ventilated hood, and add it to PBS preheated to ca. 60 °C in a beaker on a stir plate. Add 1 N NaOH dropwise until solution clears. Let solution cool to room temperature, and filter through 0.22 mm filter syringe. Adjust pH with diluted HCl to ca. 6.9, and adjust to final volume with PBS. We aliquot and freeze the solution, but it can also be stored at 4 °C for approximately four weeks.
Triton X-100	Sigma Aldrich	X100	Use wide-orifice pipette tips to handle undiluted Triton X-100.
Normal goat serum	Sigma Aldrich	G9023	To block non-specific binding of antibodies in immunofluorescence analyses.
α-CD31 Antibodies	BD Biosciences	550274	Purified rat anti-mouse monoclonal antibodies, clone MEC 13.3.
Secondary goat anti-rat antibodies	Thermo Fisher	A-11006	Goat anti-rat IgG (heavy and light chains), cross-adsorbed, fluorescently labeled with Alexa Fluor 488. Other fluorescent labels are also possible.
Mowiol 4-88	Sigma Aldrich	81381	Mounting medium for cytochemistry. MW ~31,000
Labovert	Leitz	Labovert	Inverted phase contrast microscope equipped with a 4 x and 10 x objective for somite pair counting. Other phase contrast microscopes (such as those that are routinely used in tissue culture) are also suitable.
M80	Leica Microsystems	M80	Stereomicroscope with 8 : 1 zoom range (yielding a magnification between 7.5 x and 60 x) for embryo and allantois dissection.
DM4000B	Leica Microsystems	DM4000B	Microscope system for histology with LED illumination. We use HCX PL FLUOTAR 5 x/0.15 and HC PL FLUOTAR 10 x/0.30 objectives. Other microscopes equipped phase contrast and fluorescence optics can be used to score allantois attachment.
Digital camera	JVC	KY-F75U	3-CCD digital capture camera attached to the DM4000B LED microscope. We use Diskus software (Hilgers) to operate both the microscope and the camera.
Confocal microscope	Leica Microsystems	SP5	Confocal microscope for higher- resolution imaging of endothelia in the vascular plexus of allantois explants. Immunofluorescence



	images were taken with a 40 x
	objective.
	objective.