

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 forceps 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 <sup>2+</sup> and Mg <sup>2+</sup> .  |
| 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 <sub>3</sub> 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).   |

|                                    |                    |         |   |
|------------------------------------|--------------------|---------|---|
| 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 $\mu$ m 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.   |
| $\alpha$ -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   |
| Labovet                            | Leitz              | Labovet | 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  |

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|  |  | images were taken with a 40 x objective. |
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