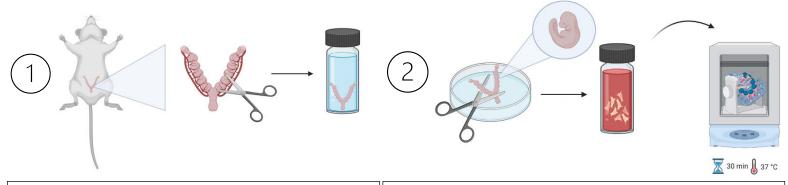
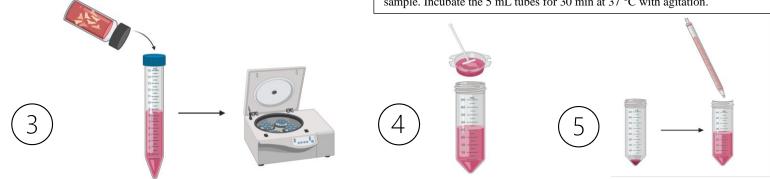
A visual guide to the main steps in the protocol

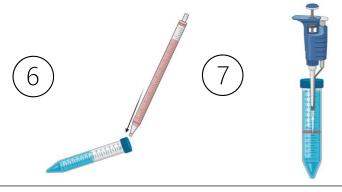


Dissect the pregnant uterus free of mesometrial fat.

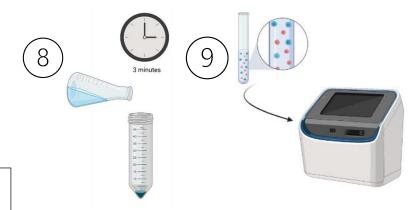
Remove the fetuses, return uterus to 5mL tube and mince tissue. Proceed with enzyme digestion step: add 3 mL of warm enzymatic digestion mix to each sample. Incubate the 5 mL tubes for 30 min at 37 $^{\circ}\text{C}$ with agitation.



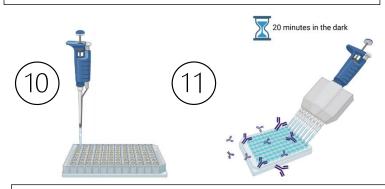
- After digestion, flush everything out of 5 mL tubes into 15 mL tubes using 10 mL of ice-cold 5mM EDTA PBS solution.
- Centrifuge 15 mL tubes containing digested tissues for 10 min at 400 x g.
- Discard the supernatant, gently flick the pellet and resuspend it in 10 mL of warm (37 °C) 5mM EDTA PBS solution.
- Incubate samples in the 15 mL tubes at 37 °C with agitation, for 15 min.
- Using the plunger of a sterile 1 mL syringe, force the digested tissue through a 70 µm strainer onto a properly labelled and sterile 50 mL tube, and spin for 10 min at 400 x g.
- After the spin, proceed with either option A (represented here in diagrams) or B. **Option A:** discard the supernatant from the 50 mL tube and using a pipet boy, resuspend each pellet in 8 mL of 40 % (v/v) isotonic Percoll in PRS



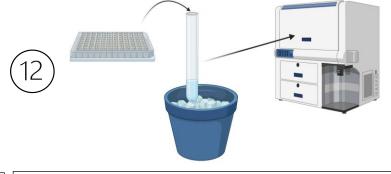
- Option A continued: Using a pipet boy on slow speed, carefully overlay the pellet resuspended in 40% Percoll solution onto the 5mL of 80 % Percoll solution. Pipette slowly and continuously; hold the 15 mL tube at an angle of 45°.
- Without disturbing the overlay, centrifuge the 15 mL tubes for 20 min at 850 x g at room temperature, acceleration 5, break 1.
- After the spin, while trying to suck a minimum amount of Percoll solution (up to 4-5 mL total), carefully collect the ring of leukocytes.



- Perform red blood cell lysis steps.
- After red blood cell lysis, perform the cell count using trypan blue and a Neubauer Chamber as per manufacturer's instructions.



- Transfer 1-2 million cells per well into a round bottom 96-well plate and proceed with spin and viability dye staining.
- After viability dye step, add 25 μL of a surface antibody cocktail.
 Proceed with further FACS steps.



Finally, transfer the samples into labelled FACS tubes and top up with 300 μ L of PBS. Keep the tubes on ice or in a fridge until processing with FACS analysis. The samples have to be run on flow cytometer within 24 h.