

## Isolation and separation

# Mouse lung endothelial cells

### Protocol for the isolation of mouse lung endothelial cells, using the gentleMACS™ Dissociator, followed by a magnetic cell sorting with CD146 (LSEC) MicroBeads

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## Background

To study the mechanisms involved in angiogenesis, transgenic mice are frequently generated. Specific isolation of endothelial cells from these mice is often necessary for phenotype, genetic, or proteomic studies. Complete dissociation of lungs into single cells is a prerequisite for reliable cell separation and cell analysis. The combination of an enzymatic digestion of the lungs and of a mechanical dissociation using the gentleMACS™ Dissociator is optimized and standardized for a high yield of leucocytes and endothelial cells, while preserving the cell surface epitopes.<sup>1</sup>

This note exemplifies the use of the gentleMACS Dissociator for the dissociation of lungs into single cells, followed by the immunomagnetic isolation of endothelial cells using CD146 (LSEC) MicroBeads.

## Materials and methods

### Materials

- Lung Dissociation Kit, mouse
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- MACSmix™ Tube Rotator in combination with an incubator at 37 °C

- Pre-Separation Filters, 30 µm and 70 µm
- CD146 (LSEC) MicroBeads, mouse
- FcR Blocking Reagent, mouse to avoid Fc receptor-mediated antibody labeling.
- MidiMACS™ Separator or QuadroMACS™ Separator
- LS Columns
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA.

### Methods

#### Lung dissociation

1. Reconstitute and prepare the reagents from the Lung Dissociation Kit according to the data sheet. Pipette enzyme mix into a gentleMACS C Tube.
2. Dissect mouse lung and rinse lobes in a petri dish containing PBS, to remove remaining blood.
3. Transfer lobes of two lungs into the gentleMACS C Tube containing the enzyme mix.
4. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **m\_lung\_01**.
6. After termination of the program, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 30 minutes at 37 °C using the MACSmix Tube Rotator. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
9. Run the gentleMACS Program **m\_lung\_02**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a 15 mL tube.

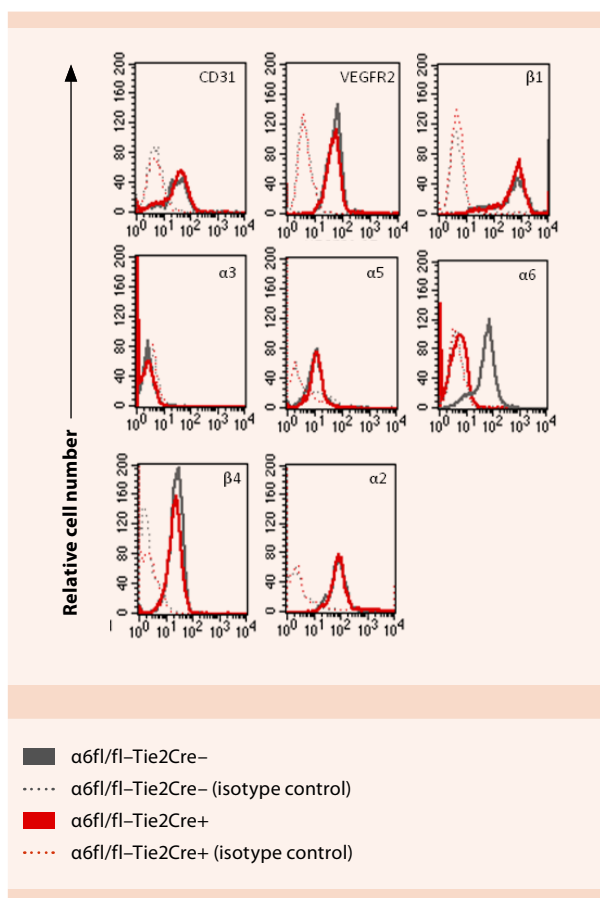
13. Wash the filter with 2.5 mL 1× Dissociation Buffer.
14. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes.
15. Aspirate supernatant completely and resuspend cells with 5 mL of PEB buffer.
16. Proceed immediately with immunomagnetic sorting.

#### Immunomagnetic sorting of CD146<sup>+</sup> cells

▲ Volumes for magnetic labeling given below are for up to 10<sup>7</sup> total cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>7</sup> total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Apply the cell suspension to a Pre-Separation Filter, 30 µm, placed on a 15 mL tube.
2. Determine cell number.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend cell pellet in 90 µL of PEB per 10<sup>7</sup> total cells.
5. Add 10 µL of FcR Blocking Reagent per 10<sup>7</sup> total cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Add 10 µL of CD146 (LSEC) MicroBeads per 10<sup>7</sup> total cells.
8. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of PEB per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10<sup>7</sup> cells in 500 µL of PEB.
11. Place LS Column in the magnetic field of a suitable MACS<sup>®</sup> Separator.
12. Prepare column by rinsing with 3 mL of PEB.
13. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
14. Wash column 3 times with 3 mL of PEB. Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
15. Remove column from the separator and place it on a 15 mL collection tube.
16. Pipette 5 mL of PEB onto the column.
17. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
18. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
19. Resuspend cells and determine cell number.

20. Cells should be processed immediately for further applications.



**Figure 1:** Representative flow cytometry analysis of lung endothelial cells from α6fl/fl-Tie2 Cre<sup>-</sup> (grey) and α6fl/fl-Tie2 Cre<sup>+</sup> (red) mice for CD31-FITC, VEGFR2-PE, and integrin subunits β1, β4, α2, α3, α5, and α6. Isotype controls are shown in dashed lines.

## Results

A high yield of leucocytes and endothelial cells could be isolated with the gentleMACS Dissociator. Cell surface epitopes are conserved which allows a complete cytometry analysis afterwards.

## Conclusion

Isolation and separation of mouse lung endothelial cells can be accomplished with ease using the gentleMACS Dissociator.

## Reference

1. Bouvard, C. *et al.* (2012) Tie2-dependent knockout of α6 integrin subunit in mice reduces post-ischaemic angiogenesis. *Cardiovasc. Res.* 95: 39–47.



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