Miltenyi Biotec

Isolation of endothelial cells from mouse liver

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1. Description

1.1 Background information

The protocol has been developed to isolate high yields of viable endothelial cells from mouse liver tissue. Cells can be cultured or analyzed by flow cytometry afterwards.

1.2 Reagent and instrument requirements

- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Liver Dissociation Kit, mouse (# 130-105-807)
- CD146 MicroBeads, mouse (# 130-092-007)
- LS Columns (# 130-042-401) and suitable MACS® Separators
- Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- Freshly prepared PEB buffer: Dilute MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222).
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
- CD31 antibodies, mouse (clone 390) conjugated to, e.g., PE
- Collagen I bovine protein (Life Technologies™, Invitrogen™)
- 0.02 M acetic acid
- 1× phosphate-buffered saline (PBS) buffer
- EBM™-2 Basal Medium (Lonza, CC-3156)
- EBM-2 Basal Medium and all supplements (EGM™-2-MV BulletKit™, Lonza, CC-3202)
- 96-well plate

2. Protocols

2.1 Sample preparation

▲ MicroBeads concentrations below are optimized for the processing of one mouse liver.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Dissociate mouse liver according to the protocol of the Liver Dissociation Kit, mouse including post-dissociation wash steps.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

2.2 Magnetic separation

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

Enrichment of CD146$^+$ cells

1. Determine cell pellet volume.
2. Add CD146 MicroBeads according to pellet volume in a 1:9 ratio, e.g., add 10 µL of CD146 MicroBeads to 90 µL pellet volume.
3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Wash cells by adding 1 mL of PEB buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
5. Place LS Column in the magnetic field of a suitable MACS Separator.

   ▲ **Note:** Automated separation can be performed by using the autoMACS Pro with the following program: Possels.
6. Prepare column by rinsing with 3 mL of PEB buffer.
7. Apply cell suspension onto the column.
8. Collect unlabeled cells that pass through. Perform three washing steps with 3 mL of PEB buffer each.
9. Collect total effluent; this is the CD146$^-$ cell fraction.
10. Remove column from the separator and place it on a suitable collection tube.
11. Pipette 5 mL of PEB buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column, this is the CD146$^+$ target cell fraction.

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2.3 Flow cytometric analysis

▲ For a detailed immunofluorescent staining protocol refer to the data sheet of the CD31 antibodies. CD31 antibodies are used due to slight blocking effects with CD146.

1. Incubate cells with CD31 antibodies.
2. Analyze cells by using a flow cytometer, e.g., the MACSQuant® Analyzer 10.

2.4 Cell culture

1. Coat of culture dish with 40 µg/cm² collagen I bovine protein (overnight at 37 °C).
   Therefore dilute collagen I bovine protein 1:50 in 0.02 M acetic acid and transfer 50 µL per 96-well. Incubate culture dish overnight at 4 °C followed by one hour incubation at room temperature. Wash culture dish with 3× 150 µL of PBS.
2. Plate 1×10^5 cells per 96-well in EBM-2 Basal Medium and all supplements.
3. After 24 hours in culture stain cells for microscope analysis.

All gentleMACS Protocols are available at **www.miltenyibiotec.com**.