Miltenyi Biotec

Isolation of endothelial cells from mouse neonatal brain

Contents

1. Description

1.1 Background information

The protocol has been developed to isolate high yields of viable endothelial cells from mouse neonatal brain 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)
- Neural Tissue Dissociation Kit (P) (# 130-092-628)
- CD45 MicroBeads, mouse (# 130-052-301)
- CD31 MicroBeads, mouse (# 130-097-418)
- LD Columns (# 130-042-901) and MS Columns (# 130-042-201) and suitable MACS® Separators
- Pre-Separation Filters, 70 µm (# 130-095-823) 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). Do not use autoMACS Running Buffer as it contains azide!
- CD31 antibodies, mouse (clone 390) conjugated to, e.g., PE (# 130-102-608)
- Fibronectin
- EBM-2 basal medium and all supplements (Lonza, EGM™-2-MV BulletKit™, CC-3202)

2. Protocols

2.1 Sample preparation

▲ MicroBeads concentrations below are optimized for the processing of 10^7 total cells from neonatal mouse brain.

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

1. Dissociate mouse neonatal brain according to the protocol of the Neural Tissue Dissociation Kit (P) including post-dissociation wash steps.
2. Determine cell number.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

2.2 Magnetic separation

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 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 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 70 µm nylon mesh (Pre-Separation Filters, 70 µm, # 130-095-823) 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.

2.2.1 Depletion of CD45$^+$ cells

1. Resuspend cells in 90 µL of PEB buffer per 10^7 total cells.
2. Add 10 µL of CD45 MicroBeads.
3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Wash cells by adding 1 mL of PEB buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
5. Place LD Column in the magnetic field of a suitable MACS Separator.

   ▲ **Note:** Automated separation can be performed by using the autoMACS® Pro or the autoMACS Separator with the following program: Depletes.

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6. Prepare column by rinsing with 3 mL of 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 $CD45^-$ fraction.
10. (Optional, if $CD45^+$ cells are needed) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of PEB buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
11. Proceed with enrichment of $CD31^+$ cells (2.2.2).

2.2.2 Enrichment of $CD31^+$ cells

1. Go on with $CD45^-$ fraction. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 90 µL of PEB buffer.
3. Add 10 µL of CD31 MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 1 mL of PEB buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. Place MS Column in the magnetic field of a suitable MACS Separator.

   ▲ **Note:** Automated separation can be performed by using the autoMACS® Pro or the autoMACS Separator with the following program: Posseld.
7. Prepare column by rinsing with 1 mL of PEB buffer:
8. Apply cell suspension onto the column.
9. Collect unlabeled cells that pass through. Perform three washing steps with 0.5 mL of PEB buffer each.
10. Collect total effluent; this is the $CD45^-/CD31^-$ cell fraction.
11. Remove column from the separator and place it on a suitable collection tube.
12. Pipette 1 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 $CD45^-/CD31^+$ target cell fraction.
13. To increase the purity of $CD31^+$ cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 6 to 12 by using a new column.

2.3 Flow cytometric analysis

▲ For a detailed immunofluorescent staining protocol refer to the data sheet of the CD31 antibodies.

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 100 µg/mL fibronectin (overnight 37 °C).
2. Plate 5×10^4 cells per 96-well in EBM-2 basal medium and all supplements.
3. After 24 hours in culture only round compact cells can be seen. Stain cells for microscope analysis at day 2.

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

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