Preparation of single-cell suspensions from mouse spleen without enzymatic treatment

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1. Description

1.1 Background information

Single-cell suspensions are a prerequisite for many experiments, for example to achieve the highest possible purity and recovery during cell separations with MACS® Technology. The gentleMACS™ Dissociator provides optimized programs to attain single-cell suspensions from various tissues, for example, mouse spleen. In combination with C Tubes, the gentleMACS Dissociator allows the automated tissue dissociation in a closed system, enabling sterile sample handling. A single tube or up to eight tubes can be processed in parallel.

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)
- Pre-Separation Filters, 30 µm, (# 130-041-407)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD).

2. Protocol for the dissociation of mouse spleen

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

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ The weight of one mouse spleen amounts to 80–120 mg (female BALB/c mouse, 6–7 weeks old).

1. Transfer mouse spleen into the gentleMACS C Tube containing buffer:

   1–2 mouse spleens: 3 mL

   3–4 mouse spleens: 6 mL

   5–6 mouse spleens: 9 mL

2. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.

3. Choose one of the gentleMACS Programs:

   1–2 mouse spleens: **m_spleen_01**.

   3–6 mouse spleens: **m_spleen_04**.

4. Run the gentleMACS Program **m_spleen_01** or **m_spleen_04**.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
6. (Optional) Perform a short centrifugation step to collect the sample material at the bottom of the tube.
7. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 30 µm, placed on a 15 mL tube (1–2 mouse spleens per C Tube) or to an appropriate cell strainer placed on a 50 mL tube (3–6 mouse spleens per C Tube).

   ▲ **Note:** Dissociated tissue can be removed from the closed C Tube by pipetting through the septum-sealed opening in the center of the cap of the C Tube. Use ART 1000 REACH 1000 µL pipette tips.

8. Wash Pre-Separation Filter with 5 mL of buffer.
9. Discard Pre-Separation Filter and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
10. Resuspend cells with buffer to the required volume for further applications.

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

140-002-293.05

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