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1. Description

1.1 Background information

The quality of single-cell suspensions derived from lymphoid tissues greatly influences the quality of the downstream applications, such as cell separation, cell analysis, or cell culture.

Mechanical as well as enzymatic methods can be used. When performing enzymatic digestions, it needs to be considered whether these may destroy cell surface antigens that are intended to be used for magnetic labeling or staining.

Enzymatic disaggregation with collagenase D is, for example, recommended to achieve optimal recovery and purity of certain cells. For details see the respective MACS® Cell Separation Reagents data sheets.

The protocols provided below are based on manual methods.

Protocols for automated tissue dissociation using the gentleMACS™ Dissociator are available at www.miltenyibiotec.com/protocols. For more details on gentle, safe and time-saving preparation of single-cell suspensions under sterile conditions please visit www.gentlemacs.com.

1.2 Reagent and instrument requirements

Reagent and instrument requirements for protocol 2.1

- 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). BSA can be replaced by other proteins such as fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- Steel mesh or nylon mesh (70 µm) cell strainer and plunger
- Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

Reagent and instrument requirements for protocol 2.2

- Collagenase D solution (2 mg/mL collagenase D (>0.15 U/mg) in 10 mM HEPES-NaOH, pH 7.4; 150 mM NaCl; 5 mM KCl; 1 mM MgCl₂; 1.8 mM CaCl₂).
- 1 mL syringe and 25G needle
- 70 µm cell strainer and plunger
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

Reagent and instrument requirements for protocol 2.3

- Pre-warmed (37 °C) plain minimum essential medium α (MEM-α) with 0.5 mg/mL collagenase D
- Complete MEM-α medium: plain MEM-α medium, 10% FCS, 10 mM HEPES, 1× penicillin/streptomycin (1:100 of a 5 mg/mL stock solution)
- 70 µm cell strainer and plunger
- Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocols

2.1 Preparation of cells by mechanical disruption

▲ This protocol is recommended for the preparation of cells from different lymphoid tissues such as mouse or rat spleen, thymus, or lymph nodes.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

1. Place tissue to be processed in a dish (6 cm in diameter) containing enough buffer (about 5 mL) to completely cover the bottom of the dish. Tease the tissue apart by using two pairs of forceps.
2. Pass the mechanically released cells and remaining tissue fragments through a steel mesh or nylon mesh by using a plunger. Collect cells in a 15 mL conical tube.
3. Wash cells by adding buffer to a final volume of about 15 mL and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend cell pellet in an appropriate amount of buffer. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps. Wet filter with buffer before use.
5. Proceed directly to downstream application. Do not store cells. For details on magnetic labeling see MACS Cell Separation Reagents data sheets.

2.2 Preparation of mouse spleen cells by enzymatic disaggregation with collagenase D

▲ This protocol is recommended for the preparation of certain cell types, for example, dendritic CD11c⁺ or mPDCA-1⁺ cells from mouse spleen using CD11c MicroBeads (# 130-052-001) or Anti-mPDCA-1 MicroBeads (# 130-091-965). For details refer to the respective data sheet at www.miltenyibiotec.com.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

1. Place isolated spleen in a dish (6 cm in diameter) containing enough collagenase D solution to completely cover the bottom of the dish (about 5 mL).
2. Inject mouse spleen with 500 µL of collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue into smaller pieces using sharp scissors.
3. Incubate spleen pieces in collagenase D solution for 30 minutes at 37 °C.
4. Pass collagenase D–released cells and remaining tissue fragments through a 70 µm cell strainer by using a plunger. Collect cells in a 15 mL tube.
5. Wash cells by adding buffer to a final volume of 14 mL and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in an appropriate amount of buffer.
7. Proceed directly to downstream application. Do not store cells. For details on magnetic labeling see MACS Cell Separation Reagents data sheets.

2.3 Preparation of cells from mouse Peyer's patches¹

▲ This protocol is recommended for the preparation of certain cell types, for example, dendritic cells, from mouse Peyer's patches (PPs).

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

1. Place freshly collected PPs in a dish containing enough pre-warmed (37 °C) plain MEM-α medium with 0.5 mg/mL collagenase D to completely cover the bottom of the dish.
2. Incubate for 15 minutes at room temperature under gentle shaking.
3. Crush PPs and pass collagenase-released cells and remaining tissue fragments through a 70 µm cell strainer by using a plunger. Collect cells in a conical tube.
4. Wash strainer with 5×2 mL of complete MEM-α medium.
5. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps. Wet filter with medium before use. Wash nylon mesh with 2×2.5 mL of complete MEM-α medium. Collect cells in a conical tube.
6. Centrifuge at 400×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Resuspend the cell pellet in an appropriate amount of buffer.

8. Proceed directly to downstream application. Do not store cells. For details on magnetic labeling see MACS Cell Separation Reagents data sheets.

3. Reference

1. Kadaoui, K. A. and Corthésy, B. (2004) Isolation of dendritic cells from mouse Peyer's patches using magnetic cell sorting. *MACS&more* 8: 10–12.

All protocols and data sheets are available at www.miltenyibiotec.com.

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