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1. Reagent and instrument requirements

- 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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- Appropriate medium.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- 10 mL syringe.
- 26-gauge needle.

2. Preparation of single-cell suspensions from mouse bone marrow

▲ All steps should be performed on ice.

1. Cut out femora and tibiae and remove the muscles.
2. Place bones into cold buffer or medium in a petri dish.
3. Collect bone marrow cells into a tube by flushing the shaft with 5 mL of buffer or medium using a 10 mL syringe with a 26-gauge needle.
4. (Optional) Repeat initial steps with next animal.
5. Disaggregate cells by gently pipetting up and down several times.
6. Pass cells through 30 µm nylon mesh (Pre-Separation Filter, # 130-041-407) to remove cell clumps. Moisten filter with buffer or medium before use.
7. Wash cells by filling up the tube with buffer or medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cells in an appropriate amount of buffer or medium for downstream applications.

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

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