Preparing Single Cell Suspensions

For safety information, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID: 214062) or the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide* (Doc ID: 214063).

Introduction

This protocol describes preparation of single cell suspensions of commonly used immune cell types. A single cell suspension can be loaded into a BD Rhapsody™ Cartridge. This protocol does not describe tissue dissociation.

Required materials

- 1X RBC Lysis Buffer (Thermo Fisher Scientific Cat. No. 00-4333-57)
- For peripheral blood mononuclear cells (PBMCs): BD Vacutainer® CPT™ Glass Mononuclear Cell Preparation Tubes–Sodium Heparin (Cat. No. 362753)
- Sample Buffer, from the BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731)

For a complete list of materials, see appropriate instrument user guide.

Before you begin

- Prepare cells as close to cartridge loading as possible.
- Some cell dissociation reagents, such as trypsin, might damage cell surface markers and decrease Sample Tag and BD™ AbSeq Ab-Oligo (antibody-oligonucleotide) sensitivity. Use cell dissociation reagents suitable for cell surface staining.
- Cells might be lost during the wash steps (25–50%). For low-abundance samples (<100,000 cells), account for cell loss when preparing single cell samples.
- Cell viability <50% might impact experimental results. If possible, use cells of high viability.
- Bead loading efficiency might be reduced in the BD Rhapsody™ Cartridge if the cell diameter is >20 µm.
- Prepare the cartridge before cell preparation. If cell preparation takes ≥4 hours, begin preparing cells before BD Rhapsody Cartridge preparation.

Isolating PBMCs using CPTs

1. Collect blood using CPTs (Cat. No. 362753), and proceed with the workflow within 2–3 hours.
2. Gently invert CPTs 8–10 times.
3. Centrifuge at 1,700 × *g* and at room temperature (15°C to 25°C) for 20 minutes.
4. Aspirate ~1/2 of the top layer containing plasma. Leave whitish mononuclear cell layer.
5. Label a new 15 mL conical tube with sample name, and gently collect mononuclear cell layer with 5 mL pipette. Transfer cells to the 15 mL tube.

   Avoid vigorous pipetting that might dislodge the gel plug in the CPT.
6. Bring volume to 10 mL with phosphate-buffered saline (PBS), and gently pipet-mix.
7. Centrifuge at 400 × *g* for 15 minutes. Aspirate supernatant.
8. Gently tap to loosen pellet.
9. Repeat steps 6–8 once for two washes.
10. Proceed to Lysing red blood cells (RBCs).

Lysing red blood cells (RBCs)

1. Add ≤1 mL PBS to the loosened pellet, and transfer suspension to a new 50 mL conical tube.
2. Pipet 10 mL 1X RBC Lysis Buffer (Thermo Fisher Scientific Cat. No. 00-4333-57) into suspension and pipet-mix.
3. Incubate at room temperature (15°C to 25°C) for 5 minutes with occasional tapping.
4. Add 20 mL PBS to the suspension, and invert tube once to mix.
5. Centrifuge at 400 × *g* for 5 minutes. Remove supernatant.
6. Loosen pellet by gently tapping.
7. Add 20 mL PBS to the suspension, and centrifuge at 400 × *g* for 5 minutes. Remove supernatant.
8. Add ≤1 mL PBS to the suspension.

9 Repeat steps 2–7 once for two lyses and washes.
10 Gently resuspend pellet in 620 µL cold Sample Buffer (Cat. No. 650000062), and place on ice.
11 Proceed to single cell capture. See the *Single Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

Preparing Jurkat and Ramos BD Rhapsody™ Training Cells

1 In laminar flow hood, label two new 15 mL conical tubes, "Jurkat" and "Ramos."
2 To each tube, pipet 8 mL PBS, and cap.
3 Gently swirl cryovials upright in 37°C water bath, ~3 minutes to thaw. Do not invert vials.
4 To Jurkat cells, add 1 mL PBS slowly drop by drop.
5 Slowly aspirate Jurkat cells and transfer to 15 mL tube with PBS. Cap.
6 Repeat steps 4–5 for Ramos cells.
7 Centrifuge tubes containing cells at 400 × *g*, 5 minutes.
8 Remove 8 mL of supernatant from each tube and pipet-mix with residual buffer.
9 Label two new 1.5 mL LoBind Tubes "Jurkat" and "Ramos."
10 Transfer resuspended cells to appropriate 1.5 mL tube.
11 Centrifuge tubes containing cells at 400 × *g*, 5 minutes.
12 Remove supernatant from each tube, and resuspend each pellet in 620 µL of cold Sample Buffer (Cat. No. 650000062), and place tubes on ice.
13 Proceed to single cell capture. See the *Single Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

Preparing cryopreserved samples, such as leukocytes or PBMCs

1 Gently swirl cryovial in 37°C water bath until cells thawed (~1–2 minutes).
2 Pipet 9 mL warm RPMI medium with 10% fetal bovine serum (FBS) into a new 15 mL conical tube.
3 Add 1 mL medium slowly, drop by drop, to thawed cells.
4 Gently pipet cells into the 15 mL conical tube containing warm RPMI medium with 10% FBS.
5 Centrifuge at 400 × *g* for 5 minutes.
6 Aspirate 9 mL supernatant, and resuspend cells in the residual 1 mL media by gently pipet-mixing.
7 Gently pipet cells into a new 1.5 mL LoBind Tube.
8 Centrifuge at 400 × *g* for 5 minutes.
9 Remove supernatant, and resuspend cells in 620 µL of cold Sample Buffer (Cat. No. 650000062), and place tube on ice.
10 Proceed to single cell capture. See the *Single Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

Troubleshooting

Observation	Possible causes	Recommended solutions
Doublets or clumps of cells	Cell samples that tend to clump	Filter the cell suspension through an appropriately sized cell strainer multiple times to remove clumps and doublets.
No pellet after centrifuging cells or very few cells	Rare or dilute sample	After each centrifugation step, leave 50 µL of supernatant.

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