BD Rhapsody™ System

Preparing Single-Cell Suspensions

Protocol

23-24126(02)
2022-11

For Research Use Only

Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
Doc ID: 210964 Rev. 1.0	2018-07	Initial release.
23-24126(01) (Doc ID: 210964 Rev. 2.0)	2021-11	Added new scomix link, and BD Rhapsody™ Enhanced Cell Capture Beads and part numbers. Removed Preparing Jurkat and Ramos section.
23-24126(02)	2022-11	Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers.

Contents

Introduction

This protocol describes the 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

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

Material	Supplier	Catalog no.
1X RBC Lysis Buffer	Thermo Fisher Scientific	00-4333-57
BD Vacutainer® CPT™ Glass Mononuclear Cell Preparation Tubes–Sodium Heparin[a]	BD Biosciences	362753
Sample Buffer from BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887[b]

a. For peripheral blood mononuclear cells (PBMCs)
b. Catalog no. for BD Rhapsody™ Enhanced Cartridge Reagent Kit

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 of <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.

Safety information

For safety information, see the *BD Rhapsody™ Single-Cell Analysis Instrument User Guide* or the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide*.

Isolating PBMCs using CPTs

1 Collect blood using CPTs, 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 (15–25 °C) for 20 minutes.
4 Aspirate ~1/2 of the top layer containing plasma. Leave the whitish mononuclear cell layer.
5 Label a new 15-mL conical tube with the sample name, and gently collect the mononuclear cell layer with a 5-mL pipette. Transfer the cells to the 15-mL tube.

Avoid vigorous pipetting that might dislodge the gel plug in the CPT.

6 Bring the volume to 10 mL with phosphate-buffered saline (PBS), and gently pipet-mix.
7 Centrifuge at 400 × *g* for 15 minutes. Aspirate the supernatant.
8 Gently tap to loosen the pellet.
9 Repeat **step 6** through **step 8** for a total of 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 the suspension to a new 50-mL conical tube.
2 Pipet 10 mL of 1X RBC Lysis Buffer into the suspension and pipet-mix.
3 Incubate at room temperature (15–25 °C) for 5 minutes with occasional tapping.
4 Add 20 mL of PBS to the suspension, and invert the tube once to mix.
5 Centrifuge at 400 × *g* for 5 minutes. Discard the supernatant.
6 Loosen the pellet by gently tapping.
7 Add 20 mL of PBS to the suspension, and centrifuge at 400 × *g* for 5 minutes. Discard the supernatant.
8 Add ≤1 mL of PBS to the suspension.
9 Repeat **step 2** through **step 7** once for two lyses and washes.
10 Gently resuspend the pellet in 620 µL of cold Sample Buffer, and place on ice.
11 Proceed to single-cell capture. See the *Single-Cell Analysis Workflow with BD Rhapsody™ Systems* or *Single-Cell Capture and cDNA Synthesis with the BD Rhapsody™ Single-Cell Analysis System*.

Preparing cryopreserved samples, such as leukocytes or PBMCs

1 Gently swirl the cryovial in a 37 °C water bath until the cells are thawed (~1–2 minutes).
2 Pipet 9 mL of warm RPMI medium with 10% fetal bovine serum (FBS) into a new 15-mL conical tube.
3 Slowly, drop-by-drop add 1 mL of medium to the thawed cells.
4 Gently pipet the 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 of supernatant, and resuspend the cells in the residual 1 mL of 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 Discard the supernatant, resuspend the cells in 620 µL of cold Sample Buffer, and place the tube on ice.
10 Proceed to single-cell capture. See the *Single-Cell Analysis Workflow with BD Rhapsody™ Systems* or *Single-Cell Capture and cDNA Synthesis with the BD Rhapsody™ Single-Cell Analysis System*.

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.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, California 95131 USA

bdbiosciences.com
scomix@bdscomix.bd.com