BD Rhapsody™ System

Single-Cell Labeling with BD® Single-Cell Multiplexing Kits

Protocol

23-21340(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 210970 Rev. 1.0	2018-07	Initial release.
Doc ID 210970 Rev. 2.0 23-21340-00	2019-02	Added BD Horizon™ Dri Tumor and Tissue Dissociation Reagent as Suggested Materials Added Mouse Immune Sample Tag sequences to Appendix A: Sequence Information.
Doc ID: 210970 Rev. 3.0 23-21340(01)	2021-11	Added BD Rhapsody™ Enhanced Cell Capture Beads and part numbers.
23-21340(02)	2022-11	Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers.

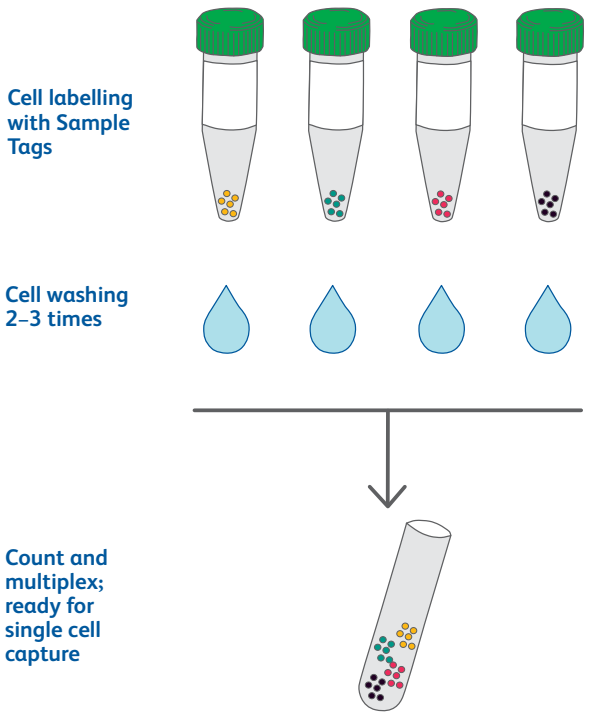
Contents

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

Introduction

The BD® Human Single-Cell Multiplexing Kit and the BD® Mouse Immune Single-Cell Multiplexing Kit utilize an innovative antibody-oligo technology to provide higher sample throughput for single-cell library preparation. Every antibody-oligo in the BD® Human Single-Cell Multiplexing Kit, referred to as a Sample Tag, has a unique sample oligo barcode conjugated to a human universal antibody and every Sample Tag in the BD® Mouse Immune Single-Cell Multiplexing Kit is conjugated to an Anti-Mouse CD45, Clone 30-F11 antibody. Up to 12 samples can be labeled and pooled prior to single-cell capture with the BD Rhapsody™ Single-Cell Analysis System.

Workflow



Required materials

Material	Supplier	Catalog no.
20,000-1 million cells	–	–
BD® Stain Buffer (FBS)	BD Biosciences	554656
BD® Human Single-Cell Multiplexing Kit[a] Or, BD® Mouse Immune Single-Cell Multiplexing Kit[a]	BD Biosciences	633781 633793
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
Latch Rack for 500-µL tubes	Thermo Fisher Scientific	4900 or 4890
Falcon® tubes, 5-mL round-bottom, polystyrene test tubes[b]	Corning	352054

a. Avoid storing Sample Tags under freezing conditions.

b. Use only the tubes specified in the protocol. Use of other tubes might lead to increased cell loss. Use only the tubes specified in the protocol. Use of other tubes might lead to increased cell loss.

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

Before you begin

- Use low retention filtered pipette tips.
- Prepare a single-cell suspension. See *Preparing Single-Cell Suspensions Protocol*.
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single-Cell Suspensions Protocol*.

Labeling with Sample Tags

1 Resuspend 20,000–1 million cells in 200 µL BD Pharmingen™ Stain Buffer (FBS).

2 Briefly centrifuge Sample Tag tubes to collect the contents at the bottom.

3 For each sample, transfer 180 µL cell suspension to a Sample Tag tube. Pipet-mix.

Caution. Aqueous buffered solution (Sample Tag) contains BSA and ≤0.1% sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

4 Incubate at room temperature (15–25 °C) for 20 minutes.

Washing Labeled Cells

Note: Sufficient post-labeling washing is important for reducing noise that comes from residual unbound antibodies being captured onto 3' capture beads during single-cell capture. However, some cell loss occurs with each additional wash. Users can choose to perform more or fewer washes depending on the abundance or their sample.

1. Transfer each labeled cell suspension to a 5-mL polystyrene Falcon® tube. Add 2 mL BD Pharmingen™ Stain Buffer to labeled cells and pipet-mix.
2. Centrifuge each tube at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.
3. Uncap each tube and invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
4. Pipet 2 mL BD Pharmingen™ Stain Buffer to each tube and resuspend by pipet-mixing.
5. Centrifuge at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.
6. Uncap each tube and invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-wiper to remove residual supernatant from tube rim.
7. (Optional) Repeat **steps 4–6** once more for a total of 3 washes.
8. Resuspend pellet in 620 µL cold Sample Buffer from the BD Rhapsody™ Enhanced Cartridge Reagent Kit. Perform viability staining and count cell using the appropriate single-cell capture and cDNA synthesis protocol.

   Note: For low-abundance samples (<20,000), resuspend the cells in 200 µL of cold BD® Sample Buffer. For other 3' single-cell capture platforms, resuspend in recommended buffer and volume according to manufacturer.
9. Place tube on ice, and proceed to single-cell capture. See the *Single-Cell Analysis Workflow with BD Rhapsody™ Systems* to find the appropriate protocol to follow.

Troubleshooting

Observation	Possible causes	Recommended solutions
Do not have the recommended buffer for labeling with Sample Tags.	Various.	Labeling with Sample Tags is optimal in BD Pharmingen™ Stain Buffer (FBS). Label Sample Tags in BD Pharmingen™ Stain Buffer (FSB).
Cells require labelling with Sample Tags at a different temperature.	Physiological requirement.	Use protocols for Sample Tag labeling that have been optimized for the specific sample type.
Accidentally resuspended cells in BD Pharmingen™ Stain Buffer (FBS) rather than Sample Buffer before cell counts.	Various.	We recommend centrifuging the samples and resuspending the cells in Sample Buffer after labeling with Sample Tags. This ensures optimal performance of cell loading in the BD Rhapsody™ Cartridge.
Cell loss.	Wrong tube used in washes.	Use Falcon® polystyrene flow tubes and centrifuge cells using a benchtop centrifuge with swing bucket rotor. This centrifugation method reduces cell loss.
Cell loss after sorting.	Various.	• Sort more cells than needed for cartridge loading. • Sort cells into 5-mL polystyrene Falcon® tube. Use the same 5-mL polystyrene Falcon® tube that was used for sorting for cell labelling by following these steps: 1. Pipet 180 µL BD Pharmingen™ Stain Buffer into each Sample Tag tube, containing 20 µL Sample Tag. 2. Pipet-mix each tube, and place on ice. 3. Sort cells into a 5-mL polystyrene Falcon® tube. 4. Centrifuge the sorted cell suspension at 400 × *g* for 5 minutes. 5. Uncap the tube and invert to decant supernatant into biohazardous waste. 6. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim. 7. Resuspend cell pellet with the 200 µL of the mix of Sample Tag and BD Pharmingen™ Stain Buffer (**step 1**), and proceed with cell labeling.

Appendix A: Sample Tag sequences

Each Human Sample Tag is a human universal antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence:

GTTGTCAAGATGCTACCGTTCAGAG[Sample Tag sequence]AAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1—Human	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2—Human	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3—Human	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT
Sample Tag 4—Human	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT
Sample Tag 5—Human	CTCCCTGGTGTTCAATACCCGATGTGGTGGGCAGAATGTGGCTGG
Sample Tag 6—Human	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC
Sample Tag 7—Human	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT
Sample Tag 8—Human	CCCCACCAGGTTGCTTTGTCGGACGAGCCCGCACAGCGCTAGGAT
Sample Tag 9—Human	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG
Sample Tag 10—Human	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC
Sample Tag 11—Human	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCCCTGCGTGC
Sample Tag 12—Human	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG

Each Mouse Immune Sample Tag is an Anti-Mouse CD45, Clone 30-F11 antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence:

GTTGTCAAGATGCTACCGTTCAGAG[Sample Tag sequence]AAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1—Mouse Immune	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCCAAG
Sample Tag 2—Mouse Immune	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC
Sample Tag 3—Mouse Immune	AGGAGGCCCCGCGTGAGAGTGATCAATCCAGGATACATTCCCGTC
Sample Tag 4—Mouse Immune	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT
Sample Tag 5—Mouse Immune	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTCGACCAGATCCT
Sample Tag 6—Mouse Immune	GCGGGCACAGCGGCTAGGGTGTTCCGGGTGGACCATGGTTCAGGC
Sample Tag 7—Mouse Immune	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC
Sample Tag 8—Mouse Immune	TCGCTGCCGTGCTTCATTGTCGCCGTTCTAACCTCCGATGTCTCG
Sample Tag 9—Mouse Immune	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC
Sample Tag 10—Mouse Immune	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC
Sample Tag 11—Mouse Immune	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGACTCGCGT
Sample Tag 12—Mouse Immune	CTGGGTGCCTGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC

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