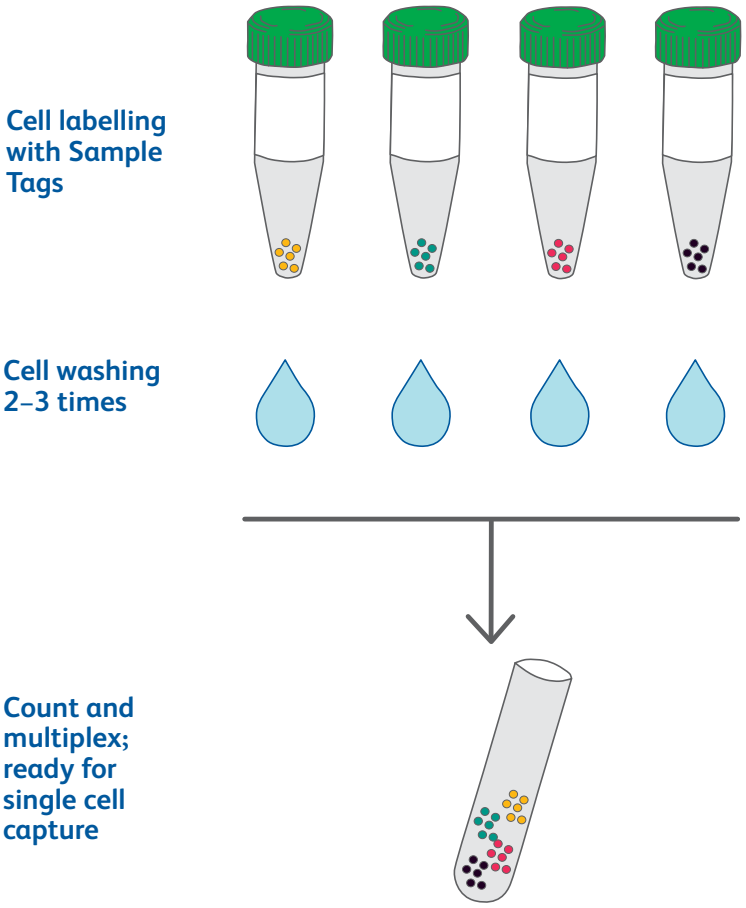
Single Cell Labelling with the BD™ Single-Cell Multiplexing Kits

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 User Guide* (Doc ID: 214063).

Introduction

The BD™ Human Single-Cell Multiplexing Kit (Cat. No. 633781) and the BD™ Mouse Immune Single-Cell Multiplexing Kit (Cat. No. 633793) 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 labelled and pooled prior to single cell capture with the BD Rhapsody™ Single-Cell Analysis system.

Workflow



Required materials

- 20,000–1 million cells
- BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656)
- BD Human Single-Cell Multiplexing Kit (Cat. No. 633781) or BD Mouse Immune Single-Cell Multiplexing Kit (Cat. No. 633793)
  Never freeze Sample Tags.
- BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731)
- 5 mL polystyrene Falcon® tube (Corning Cat. No. 352054)
  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.

Suggested materials

- BD Horizon™ Dri Tumor and Tissue Dissociation Reagent (Contact your local sales representative to purchase the product.)

Other methods may be compatible but they must be nuclease free to ensure Sample Tag performance.

Before you begin

- Use low retention filtered pipette tips.
- Prime and treat BD Rhapsody™ Cartridge. See appropriate instrument user guide.
- Prepare a single cell suspension. See *Preparing Single Cell Suspensions Protocol* (Doc ID: 210964).
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single Cell Suspensions Protocol* (Doc ID: 210964).

Labelling cells with Sample Tags

1 Resuspend 20,000–1 million cells in 200 µL BD Pharmingen Stain Buffer (FBS) (Cat. No. 554656).

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. (See 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:.) 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°C to 25°C) for 20 minutes.

Washing Labelled Cells

Note: Sufficient post-labelling washes are 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 labelled cell suspension to a 5 mL polystyrene Falcon tube (Corning Cat. No. 352054), and pipet 2 mL BD Pharmingen Stain Buffer into labelled cells and pipet-mix.

2 Centrifuge each tube at 400 × *g* for 5 minutes.

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 (FBS) into each tube, and resuspend by pipet-mixing.

5 Centrifuge at 400 × *g* for 5 minutes.

6 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.

7 (Optional) Repeat steps 4–6 once more for a total of 3 washes.

8 Resuspend pellet in 620 µL cold Sample Buffer (Cat. No. 650000062) from the BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731). 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* (Doc ID: 220524) to find the appropriate protocol to follow.

Troubleshooting

Observation	Possible causes	Recommended solutions
Do not have the recommended buffer for labelling with Sample Tags	Various	Labelling with Sample Tags is optimal in BD Pharmingen Stain Buffer (FBS) (Cat. No. 554656). Label Sample Tags in BD Pharmingen Stain Buffer (FBS).
Cells require labelling with Sample Tags at a different temperature	Physiological requirement	Use protocols for Sample Tag labelling 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	BD Biosciences recommends centrifuging the samples and resuspending the cells in Sample Buffer after labelling 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 the 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 labelling.

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

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA
Tel 1.877.232.8995

bdbiosciences.com
ResearchApplications@bd.com

History

Revision	Date	Changes made
Doc ID 210970 Rev. 1.0	07/2018	Initial release.
Doc ID: 210970 Rev. 2.0 23-21340-00	02/2019	• Added BD Horizon Dri Tumor and Tissue Dissociation Reagent as Suggested Materials • Added Mouse Immune Sample Tag sequences to Appendix A: Sequence Information.