 **BD Rhapsody™ System**  
Single-Cell Labeling with  
BD® Flex Single-Cell Multiplexing Kits  
Protocol

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## Regulatory information

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

## History

Revision	Date	Change made
23-24311(01)	2022-12	Initial release.

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

## Introduction

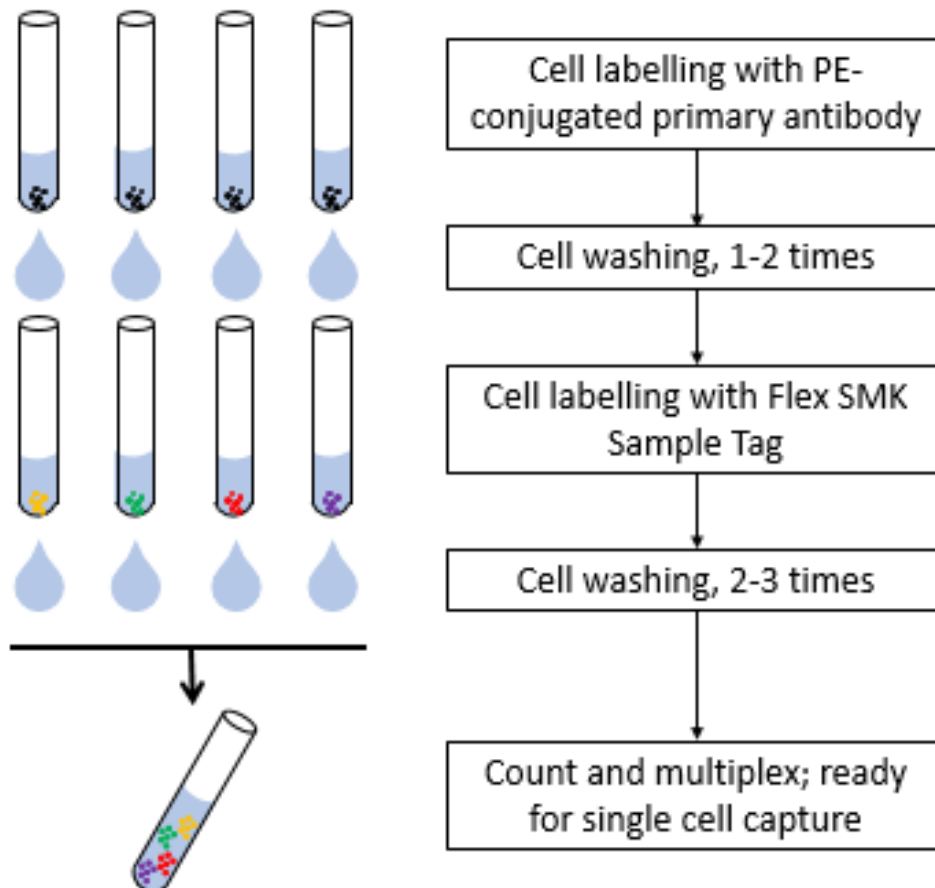
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BD® Flex Single-Cell Multiplexing Kits utilize an innovative antibody-oligo technology to provide higher sample throughput for single-cell library preparation, as well as improve detection and elimination of multiplet data from single-cell experiments. Every antibody-oligo in the BD® Flex Single-Cell Multiplexing Kits, referred to as a Sample Tag, has a unique sample oligo barcode conjugated to an Anti-R-Phycoerythrin (PE), Clone E31-1459 antibody. This approach allows users to select any PE-conjugated primary antibody to tag their samples, regardless of species or cell type.

Four BD® Flex Single-Cell Multiplexing Kits (Cat. no. 633849-633852) are available, each consisting of six Sample Tags with unique sample oligo barcodes. These four kits are fully compatible, allowing up to 24 samples to be labeled and pooled prior to single-cell capture with the BD Rhapsody™ HT Single-Cell Analysis system.

## Workflow

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## Required materials

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- 50,000–1 million cells
- BD Pharmingen™ Stain Buffer (FBS) (Cat. no. 554656)
- PE-conjugated primary antibody (specific to sample of interest)
- BD® Flex Single-Cell Multiplexing Kits (Cat. no. 633849-633852)

**Note:** Never freeze BD® AbSeq Ab-Oligos or Sample Tags.

- BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. no. 664887)
- Falcon® tubes, 5-mL Round Bottom Polystyrene Test Tube (Corning Cat. no. 352054)

**Note:** 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 the instrument user guide.

## Suggested materials

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- BD Pharmingen™ Human BD Fc Block™ (Cat. no. 564219)
- BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) (Cat. no. 553141)

Or,

BD Pharmingen™ Purified Mouse Anti-Rat CD32 (Rat BD Fc Block™) (Cat. no. 550270)

## Before you begin

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- 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*.
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single-Cell Suspensions Protocol*.

## Cell labeling with PE-conjugated primary antibody

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1. For each sample, prepare single-cell suspension.
2. 50,000 – 1 million cells per sample in 5-mL Round Bottom Polystyrene Falcon® Test Tubes.
3. Centrifuge cells at 400 × g for 5 minutes.
4. (Optional) For samples containing myeloid and B lymphocytes, we recommend blocking non-specific Fc Receptor mediated false-positive signal with Human BD Fc Block™, BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™), or BD Pharmingen™ Purified Mouse Anti-Rat CD32 (Rat BD Fc Block™).

To perform blocking:

- a. Pipet reagents into a new 1.5-mL LoBind tube on ice:

### BD Fc Block™ MasterMix

Component	For 1 sample (μL) <sup>a</sup>	For 1 sample + 20% overage (μL)
BD Pharmingen™ Stain Buffer (FBS)	95.0	114.0
BD Pharmingen™ Human BD Fc Block™ <sup>b</sup>	5.0	6.0
<b>Total</b>	<b>100.0</b>	<b>120.0</b>

a. Sufficient for  $\leq 1 \times 10^6$  cells. To block more cells, adjust volume.

b. Depending on the cell type, substitute BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™), or BD Pharmingen™ Purified Mouse Anti-Rat CD32 (Rat BD Fc Block™).

- b. Pipet-mix BD Fc Block™ MasterMix and briefly centrifuge. Place on ice.
  - c. Remove supernatant from cells without disturbing pellet.
  - d. Resuspend cells in 100 μL BD Fc Block™ MasterMix.
  - e. Incubate at room temperature (15–25 °C) for 10 minutes.
  - f. After BD Fc Block™, proceed to **step 6**.
5. Remove supernatant from cells without disturbing pellet and resuspend each sample in 100 μL of BD Pharmingen™ Stain Buffer (FBS). Pipet-mix.
  6. Stain cells with PE-conjugated primary antibody following supplier's recommended staining protocol.

**Note:** Selection of primary PE-conjugated antibody and staining concentration can impact the performance of BD® Flex SMK. Ideally the target(s) will be uniformly expressed at high levels on all of the cell types in your sample. Expression level and optimal titration of antibody on the sample of interest can be predetermined by flow cytometry.

7. Add 2 mL of BD Pharmingen™ Stain Buffer (FBS) to each tube and resuspend by pipet-mixing.

8. Centrifuge at  $400 \times g$  for 5 minutes.
9. 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.
10. (Optional) Repeat **steps 7–9** once more for a total of 2 washes.
11. Resuspend cells in 180  $\mu\text{L}$  of BD Pharmingen™ Stain Buffer (FBS) and place on ice.

## Labeling single cells with Sample Tags

1. Briefly centrifuge Sample Tag tubes to collect the contents at the bottom.
2. For each sample, transfer 20  $\mu\text{L}$  Sample Tag to cell suspension. Pipet-mix.



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

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

## Washing labeled cells

**Note:** Sufficient post-labeling 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. Add 2 mL BD Pharmingen™ Stain Buffer (FBS) to labeled cells and pipet-mix.
2. Centrifuge each tube at  $400 \times g$  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. Add 2 mL BD Pharmingen™ Stain Buffer (FBS) to each tube and resuspend by pipet-mixing.
5. Centrifuge at  $400 \times g$  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  $\mu\text{L}$  cold Sample Buffer from the BD Rhapsody™ Cartridge Reagent Kit. Perform viability staining and count cell using the appropriate single-cell capture and cDNA synthesis protocol.

**Note:** For low-abundance samples (<50,000), resuspend the cells in 200  $\mu\text{L}$  of cold BD Sample Buffer.

We recommend pooling more cells (up to one million cells) than you want to be captured in the BD Rhapsody™ Cartridge, because there can be cell loss during BD® Flex SMK labeling and washing.

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.

## Appendix A: Sample Tag sequences

Each Flex Sample Tag is an anti-PE 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]AAAAAAAAAAAAAAAAAAAAAAAAAAAA

### BD® Flex Single-Cell Multiplexing Kit A (Cat. No. 633849)

**Note:** Not Compatible with Hu SMK Tags 1-6 (Cat. No. 633781)

Sample Tag	Sample Tag Sequence	Notes
Sample Tag 1 – Flex	ATCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG	Barcode sequence is the same as human SMK Sample Tag 1
Sample Tag 2 – Flex	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG	Barcode sequence is the same as human SMK Sample Tag 2
Sample Tag 3 – Flex	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAACTCCGTGTATCCT	Barcode sequence is the same as human SMK Sample Tag 3
Sample Tag 4 – Flex	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT	Barcode sequence is the same as human SMK Sample Tag 4
Sample Tag 5 – Flex	CTCCCTGGTGTTC AATACCCGATGTGGTGGGCAGAATGTGGCTGG	Barcode sequence is the same as human SMK Sample Tag 5
Sample Tag 6 – Flex	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC	Barcode sequence is the same as human SMK Sample Tag 6

### BD® Flex Single-Cell Multiplexing Kit B (Cat. No. 633850)

**Note:** Not Compatible with Hu SMK Tags 7-12 (Cat. No. 633781)

Sample Tag	Sample Tag Sequence	Notes
Sample Tag 7 – Flex	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT	Barcode sequence is the same as human SMK Sample Tag 7
Sample Tag 8 – Flex	CCCCACCAGGTTGCTTTGTCTGGACGAGCCCGCACAGCGCTAGGAT	Barcode sequence is the same as human SMK Sample Tag 8
Sample Tag 9 – Flex	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG	Barcode sequence is the same as human SMK Sample Tag 9
Sample Tag 10 – Flex	GCAGCCGGCGTCTGACGAGGCACAGCGGAGACTAGATGAGGCCCC	Barcode sequence is the same as human SMK Sample Tag 10
Sample Tag 11 – Flex	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCTGCGTGC	Barcode sequence is the same as human SMK Sample Tag 11
Sample Tag 12 – Flex	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG	Barcode sequence is the same as human SMK Sample Tag 12



**BD® Flex Single-Cell Multiplexing Kit C (Cat. No. 633851)****Note:** Not Compatible with Ms SMK Tags 1-6 (Cat. No. 633793)

Sample Tag	Sample Tag Sequence	Notes
Sample Tag 13 – Flex	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCAAG	Barcode sequence is the same as mouse SMK Sample Tag 1
Sample Tag 14 – Flex	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC	Barcode sequence is the same as mouse SMK Sample Tag 2
Sample Tag 15 – Flex	AGGAGGCCCGCGTGAGAGTGATCAATCCAGGATACATTCCCCTC	Barcode sequence is the same as mouse SMK Sample Tag 3
Sample Tag 16 – Flex	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT	Barcode sequence is the same as mouse SMK Sample Tag 4
Sample Tag 17 – Flex	GGCAAGGTGTACATTGGGCTACCGCGGGAGGTCGACCAGATCCT	Barcode sequence is the same as mouse SMK Sample Tag 5
Sample Tag 18 – Flex	GCGGGCACAGCGGCTAGGGTGTTCCGGGTGGACCATGGTTCAGGC	Barcode sequence is the same as mouse SMK Sample Tag 6

**BD® Flex Single-Cell Multiplexing Kit D (Cat. No. 633852)****Note:** Not Compatible with Ms SMK Tags 7-12 (Cat. No. 633793)

Sample Tag	Sample Tag Sequence	Notes
Sample Tag 19 – Flex	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC	Barcode sequence is the same as mouse SMK Sample Tag 7
Sample Tag 20 – Flex	TCGCTGCCGTGCTTCATTGTGCGCGTTCTAACCTCCGATGTCTCG	Barcode sequence is the same as mouse SMK Sample Tag 8
Sample Tag 21 – Flex	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC	Barcode sequence is the same as mouse SMK Sample Tag 9
Sample Tag 22 – Flex	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC	Barcode sequence is the same as mouse SMK Sample Tag 10
Sample Tag 23 – Flex	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGAAGTCCGCT	Barcode sequence is the same as mouse SMK Sample Tag 11
Sample Tag 24 – Flex	CTGGGTGCCTGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC	Barcode sequence is the same as mouse SMK Sample Tag 12

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