BD Rhapsody™ System

Single-Cell Labeling with BD® AbSeq Ab-Oligos

(from 41 plex to 100 plex)

For Research Use Only

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

bdbiosciences.com
scomix@bdscomix.bd.com

Regulatory Information

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

History

Revision	Date	Change made
23-22314-00	2019-12	Initial release
23-22314(01)	2021-11	Added Enhanced Cell Capture Beads and part numbers.

Contents

Introduction

This protocol describes the use of BD® AbSeq Ab-Oligos (antibody-oligonucleotides) for antigen expression profiling with BD Rhapsody™ single-cell capture and downstream library preparation. Each BD® AbSeq Ab-Oligo is an oligonucleotide-conjugated antibody that contains an antibody-specific barcode and poly(A) tail for bead capture, PCR amplification, and library generation. The protocol supports the BD® AbSeq Ab-Oligo labeling of 20,000 to 1 million cells. Up to 100 antibodies can be pooled together per staining reaction. This protocol is specific for pools of greater than 40 Ab-Oligos.

Required materials

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

NOTE Use only the tubes specified in the protocol. Use of other tubes could lead to sub-optimal results.

- 20,000–1 million cells
- BD® Stain Buffer (FBS) [Cat. No. 554656]
- BD® AbSeq Ab-Oligos (various Cat. Nos.)

  Avoid storing BD® AbSeq Ab-Oligos under freezing conditions.
- BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731) or BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. No. 664887)
- Latch Rack for 500-µL tubes (Thermo Fisher Scientific, Cat. Nos. 4900 or 4890)
- Falcon® tubes, 5-mL round-bottom, polystyrene test tube (Corning, Cat. No. 352054)

Suggested materials

- Human BD Fc Block™ (Cat. No. 564220)
- 8-Channel Screw Cap Tube Capper (Thermo Fisher Scientific, Cat. No. 4105MAT)
- Multi-channel pipette

Before you begin

- Use low-retention filtered pipette tips.
- Prime and treat the BD Rhapsody™ Cartridge. See the appropriate BD Rhapsody™ 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).

Safety information

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

Preparing BD® AbSeq labeling MasterMix

BD Biosciences recommends:

- Creating freshly pooled antibody-oligos before each experiment.
- Creating AbSeq Ab-Oligo pools with 30% overage to ensure adequate volumes for labeling.

  NOTE The reagents are viscous and can form bubbles easily. The reagents are provided with sufficient volumes to cover 30% overage.

  For pooling a large number of AbSeq Ab-Oligos, using an 8-Channel Screw Cap Tube Capper and multi-channel pipette to pipet BD® AbSeq Ab-Oligos into 8-tube strips is recommended. Centrifuge the tube strip prior to pooling BD® AbSeq Ab-Oligos into a 1.5-mL LoBind tube as outlined below.

1. Place all tubes of BD® AbSeq Ab-Oligos to be pooled into a Latch Rack for 500-µL tubes (Thermo Fisher Scientific, Cat. No. 4890). Arrange the tubes so that they can be easily uncapped and re-capped with an 8-Channel Screw Cap Tube Capper (Thermo Fisher Scientific, Cat. No. 4105MAT) and aliquoted with a multi-channel pipette.
2. Centrifuge the BD® AbSeq Ab-Oligo tubes in the Latch Rack in a tabletop centrifuge with a plate adapter at 400 × g for 30 seconds prior to uncapping and then place on ice.
3. In the pre-amplification workspace, pipet the reagents into a new 1.5-mL LoBind tube on ice.

BD® AbSeq labeling MasterMix

For 87 plex and below, if optional Fc Block™ is performed, adjust the total volume to 175 µL. If performing 88 plex and above, do not adjust volumes. In this case, the total stain volume will be 225 µL if optional Fc Block™ is performed. See the following examples.

Component	For 1 sample (µL)	For 1 sample + 30% overage (µL)	For 2 samples + 30% overage (µL)
Per BD® AbSeq Ab-Oligo	2.0	2.6	5.2
Total	2.0 * *N*	2.6 * *N*	5.2 * *N*
BD® Stain Buffer (FBS) (Cat. No. 554656)	200.0 – (2.0 * *N*)	260 – (2.6 * *N*)	520 – (5.2 * *N*)
Total	**200.0**	**260**	**520**

N = no. of antibodies

Examples of different pools of AbSeq Ab-Oligos are described below:

Component	For 1 sample (µL)	For 1 sample + 30% overage (µL)	For 2 samples + 30% overage (µL)
60-plex BD® AbSeq labeling without Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (120.0 total)	2.6 (156.0 total)	5.2 (312.0 total)
BD® Stain Buffer (FBS) (Cat. No. 554656)	80	104	208
Total	**200**	**260**	**520**
60-plex BD® AbSeq labeling with Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (120.0 total)	2.6 (156.0 total)	5.2 (312.0 total)
BD® Stain Buffer (FBS) (Cat. No. 554656)	55	71.5	143
Total	**175**	**227.5**	**455**
90-plex BD® AbSeq labeling with or without Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (180.0 total)	2.6 (234.0 total)	5.2 (468.0 total)
BD® Stain Buffer (FBS) (Cat. No. 554656)	20	26	52
Total	**200**	**260**	**520**

4 Pipet-mix the AbSeq labeling MasterMix and place back on ice.

Labeling cells with BD® AbSeq Ab-Oligos

1 Centrifuge the cells at 400 × g for 5 minutes.

2 (Optional) For samples containing myeloid and B lymphocytes, BD Biosciences recommends blocking non-specific Fc Receptor–mediated false-positive signal with Human BD Fc Block™ (Cat. No. 564220).

To perform blocking:

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

Fc Block™ MasterMix

Component	For 1 sample (µL)[a]	For 1 sample + 20% overage (µL)
BD® Stain Buffer (FBS) (Cat. No. 554656)	20.0	24.0
Human BD Fc Block™ (Cat. No. 564220)	5.0	6.0
Total	**25.0**	**30.0**

a. Sufficient for ≤1 × 10^6 cells. To block more cells, adjust the volume.

b Pipet-mix the Fc Block™ MasterMix and briefly centrifuge. Place on ice.

c Remove the supernatant from the cells without disturbing the pellet.

d Resuspend the cells in 25 µL of Fc Block™ MasterMix.

e Incubate the cells at room temperature (15°C to 25°C) for 10 minutes.

f Add 175 µL (87 plex and below) or 200 µL (88 plex and above) of BD® AbSeq labeling MasterMix into the cell suspension. Pipet-mix and proceed to step 4.

3 Remove the supernatant from the cells without disturbing the pellet, and resuspend with 200 µL of BD® AbSeq labeling MasterMix. Pipet-mix.

4 Transfer the cells and AbSeq labeling MasterMix (200–225 µL) into a new 5-mL polystyrene Falcon® tube.

5 Incubate on ice for 30–60 minutes.

NOTE If the staining volume exceeds 200 µL, incubation times longer than 30 minutes may increase sensitivity.

Washing labeled cells

NOTE Sufficient post-labeling washes are important for reducing noise that comes from residual unbound Ab-Oligos being captured onto 3' capture beads during single-cell capture. However, some cell loss occurs with each additional wash. You can choose to perform more or fewer washes depending on the cell abundance.

1. Add 3 mL of BD® Stain Buffer to labeled cells and pipet-mix for dilution.
2. Centrifuge each tube at 400 × g for 5 minutes.
3. Uncap each tube and invert to decant the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove the residual supernatant from the tube rim.
4. Add 3 mL of BD® Stain Buffer to each tube and resuspend by pipet-mixing for the first wash.
5. Centrifuge at 400 × g for 5 minutes.
6. Uncap each tube and invert to decant the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove the residual supernatant from the tube rim.
7. (Optional) Repeat step 4 through step 6 once or twice more for a total of two to three washes.
8. Resuspend the pellet in 620 µL of cold Sample Buffer (Cat. No. 650000062) from the BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731) or BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. No. 664887). Perform viability staining and count the cells 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 the recommended buffer and volume according to the manufacturer.
9. Place the 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 labeling with BD® AbSeq Ab-Oligos	Various	Labeling with BD® AbSeq Ab-Oligos has been optimized in BD® Stain Buffer (FBS). Use of other staining buffers could result in less than optimal staining.
Total stain volume exceeds 225 µL	Staining with ≥100 BD® AbSeq Ab-Oligos	Staining with ≥100 BD® AbSeq Ab-Oligos requires that no BD® Stain Buffer (FBS) is used in order to keep the staining volume between 200–225 µL. Staining with ≥100 BD® AbSeq Ab-Oligos results in >200–225 µL (Fc Block™) stain volume may require incubation up to 60 minutes on ice for optimal results.
Cells require labeling with BD® AbSeq Ab-Oligos at a different temperature	Physiological requirement	Protocols for BD® AbSeq Ab-Oligo labeling have been optimized for staining on ice. Use of other staining temperatures has not been tested and requires user optimization. For certain cytokine receptors, staining at room temperature can increase the sensitivity. However, incubation at room temperature for long periods of time might negatively impact the cell viability and RNA quality.
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.
	Excessive washing or loss during washing	We recommend washing cells at least twice before loading onto the BD Rhapsody™ cartridge to decrease noise caused by unbound Ab-Oligos. Fewer washes can cause higher levels of noise. Therefore, for certain cell types, user optimization is required for the washing step to minimize cell loss and to avoid high levels of noise.
Cell loss during sorting	Various	• Sort more cells than needed for cartridge loading. • Label BD® AbSeq and fluorescent antibody together to reduce cell loss during multiple washing steps, and proceed to cartridge loading right after sorting.