BD Rhapsody™ System

Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex)

Protocol

23-24262(01)
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: 214394 Rev. 1.0	2018-07	Initial release.
23-24262(01)	2022-11	Added BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers.

Contents

Introduction

This protocol describes 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 BD® AbSeq Ab-Oligo labeling of 20,000 to 1 million cells. Up to 40 antibodies can be pooled together per staining reaction. For greater than 40 antibodies, refer to *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex) Single-Cell Protocol*.

Required materials

Material	Supplier	Catalog no.
20,000-1 million cells	–	–
BD® Stain Buffer (FBS)	BD Biosciences	554656
BD® AbSeq Ab-Oligos[a]	BD Biosciences	Various
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 BD® AbSeq Ab-Oligos or Sample Tags under freezing conditions.

b. 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

Material	Supplier	Catalog no.
Human BD Fc Block™	BD Biosciences	564220
Or,		
Mouse BD Fc Block™	BD Biosciences	553142
8-Channel Screw Cap Tube Capper	Thermo Fisher Scientific	4105MAT
Multi-channel pipette	Major supplier	–

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*.

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*.

Preparing 2X BD® AbSeq antibody-oligo labeling MasterMix

We recommend:

- Creating freshly pooled antibodies before each experiment.
- Creating pools with 30% overage to ensure adequate volumes for labeling. The reagents are viscous and form bubbles easily.
- For high-plex panels, using an 8-Channel Screw Cap Tube Capper and multi-channel pipette to pipet BD® AbSeq Ab-Oligos into 8-tube strips. Centrifuge tube strip and pool BD® AbSeq Ab-Oligos into a 1.5-mL LoBind tube.

1 Place all BD® AbSeq Ab-Oligos to be pooled into a Latch Rack for 500 µL tubes. Arrange the tubes so that they can be easily uncapped and re-capped with an 8-Channel Screw Cap Tube Capper and aliquoted with a multi-channel pipette.

2 Centrifuge BD® AbSeq Ab-Oligos in the Latch Rack in a tabletop centrifuge with a plate adapter tubes at 400 × *g* for 30 seconds and place on ice.

3 In pre-amplification workspace, pipet reagents into a new 1.5-mL LoBind Tube on ice:

2X BD® AbSeq labeling MasterMix

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
Per BD® AbSeq Ab-Oligo	2.0	2.6	5.2
BD Stain Buffer (FBS) (*N* = no. antibodies)	100 – (2.0 × *N*)	130 – (2.6 × *N*)	260 – (5.2 × *N*)
Total	**100.0**	**130.0**	**260.0**

Examples

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
10-plex BD® AbSeq labeling			
Per BD® AbSeq Ab-Oligo	2.0 (20.0 total)	2.6 (26.0 total)	5.2 (52.0 total)
BD Stain Buffer (FBS)	80.0	104.0	208.0
20-plex BD® AbSeq labeling			
Per BD® AbSeq Ab-Oligo	2.0 (40.0 total)	2.6 (52.0 total)	5.2 (104.0 total)
BD Stain Buffer (FBS)	60.0	78.0	156.0
40-plex BD® AbSeq labeling			
Per BD® AbSeq Ab-Oligo	2.0 (80.0 total)	2.6 (104.0 total)	5.2 (208.0 total)
BD Stain Buffer (FBS)	20.0	26.0	52.0

4 Pipet-mix the 2X BD® AbSeq labeling MasterMix, and place back on ice.

Labeling cells with BD® AbSeq Ab-Oligos

1 Centrifuge cells at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.

2 (Optional) For samples containing myeloid and B lymphocytes, we recommend blocking non-specific Fc Receptor-mediated false-positive signal with Human BD Fc Block™ or Mouse BD Fc Block™, as appropriate.

To perform blocking:

a Pipet 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)	95.0	114.0
Human BD Fc Block™ or Mouse BD Fc Block™	5.0	6.0
Total	**100.0**	**120.0**

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

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

c Discard supernatant from cells without disturbing pellet.

d Resuspend cells in 110 µL Fc Block™ MasterMix.

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

f After Fc Block™, proceed to **step 4**.

3 Discard supernatant from cells without disturbing pellet, and resuspend each sample in 110 µL BD® Stain Buffer. Pipet-mix.

4 In a new 5-mL polystyrene Falcon® tube, combine 100 µL of cell suspension and 100 µL 2X BD® AbSeq labeling MasterMix. Pipet-mix

5 Incubate on ice for 30–60 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 of their sample.

1. Add 2 mL BD® 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. Add 2 mL BD® 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-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 the 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 BD® AbSeq Ab-Oligos.	Various.	Labeling with BD® AbSeq Ab-Oligos is optimal in BD® Stain Buffer (FBS). Label BD® AbSeq Ab-Oligos in BD® Stain Buffer (FBS).
Cells require labeling with BD® AbSeq Ab-Oligos at a different temperature.	Physiological requirement	Use protocols for BD® AbSeq Ab-Oligo labeling that have been optimized for the specific sample type.
Accidentally resuspended cells in BD® 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 BD® AbSeq Ab-Oligos. 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 labeling by following these steps: 1. Create a 1X AbSeq labeling MasterMix by adding 100 µL BD® Stain Buffer per 100 µL 2X BD® AbSeq labeling MasterMix. 2. Pipet-mix, 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 1X BD® AbSeq labeling MasterMix (**step 1**), and proceed with cell labeling.

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