Single Cell Labelling with BD™ AbSeq Ab-Oligos

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 Instrument User Guide* (Doc ID: 214063).

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 labelling of 20,000 to 1 million cells. Up to 40 antibodies can be pooled together per staining reaction.

Required materials

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

  Never freeze BD AbSeq Ab-Oligos.
- BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731)
- Latch Rack for 500 µL Tubes (Thermo Fisher Scientific Cat. No. 4900 or 4890)
- Falcon® tubes, 5 mL Round Bottom Polystyrene Test 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

- 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 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).

Preparing 2X BD AbSeq antibody-oligo labelling master mix

Note: BD Biosciences recommends:

- Creating freshly pooled antibodies before each experiment.
- Creating pools with 30% overage to ensure adequate volumes for labelling. **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 (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 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 labelling master mix

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) (Cat. No. 554656) (*N* = no. antibodies)	100.0 – (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 labelling			
Per BD AbSeq Ab-Oligo	2.0 (20.0 total)	2.6 (26.0 total)	5.2 (52.0 total)
BD Stain Buffer (FBS) (Cat. No. 554656)	80.0	104.0	208.0
20-plex BD AbSeq labelling			
Per BD AbSeq Ab-Oligo	2.0 (40.0 total)	2.6 (52.0 total)	5.2 (104.0 total)
BD Stain Buffer (FBS) (Cat. No. 554656)	60.0	78.0	156.0
40-plex BD AbSeq labelling			
Per BD AbSeq Ab-Oligo	2.0 (80.0 total)	2.6 (104.0 total)	5.2 (208.0 total)
BD Stain Buffer (FBS) (Cat. No. 554656)	20.0	26.0	52.0

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

Labelling cells with BD AbSeq Ab-Oligos

1 Centrifuge 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 reagents into a new 1.5 mL LoBind Tube on ice:

Fc Block master mix

Component	For 1 sample (µL)[a]	For 1 sample + 20% overage (µL)
BD Stain Buffer (FBS) (Cat. No. 554656)	95.0	114.0
Human BD Fc Block (Cat. No. 564220)	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 master mix and briefly centrifuge. Place on ice.

c. Remove supernatant from cells without disturbing pellet.

d. Resuspend cells in 110 µL Fc block master mix.

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

f. After Fc Block, proceed to step 4.

3 Remove supernatant from cells without disturbing pellet, and resuspend each sample in 110 µL BD Stain Buffer (Cat. No. 554656). Pipet-mix.

4 In new 5 mL polystyrene Falcon tube (Corning Cat. No. 352054), combine 100 µL of cell suspension and 100 µL 2X BD AbSeq labelling master mix. Pipet-mix.

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

1 Add 2 mL BD Stain Buffer to 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 Add 2 mL BD Stain Buffer to 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 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 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 BD AbSeq Ab-Oligos	Various	Labelling with BD AbSeq Ab-Oligos is optimal in BD Stain Buffer (FBS) (Cat. No. 554656). Label BD AbSeq Ab-Oligos in BD Stain Buffer (FBS).
Cells require labelling with BD AbSeq Ab-Oligos at a different temperature	Physiological requirement	Use protocols for BD AbSeq Ab-Oligo labelling 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	BD Biosciences recommends centrifuging the samples and resuspending the cells in Sample Buffer after labelling 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 labelling by following these steps: 1. Create a 1X AbSeq labelling master mix by adding 100 µL BD Stain Buffer per 100 µL 2X BD AbSeq labelling master mix. 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 labelling master mix (step 1), and proceed with cell labelling.

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Doc ID: 214394 Rev. 1.0