# BD Rhapsody™ System

## Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex)

Protocol

23-22314(02)
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 |
|---|---|---|
| 23-22314-00 | 2019-12 | Initial release. |
| 23-22314(01) | 2021-11 | Added BD Rhapsody™ Enhanced Cell Capture Beads and part numbers. |
| 23-22314(02) | 2022-11 | Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed 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-Oligos 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-Oligos 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.

| 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. | | |

# 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 BD® AbSeq labeling MasterMix

We recommend:

- Creating freshly pooled antibody-oligos before each experiment.
- Creating BD® 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 BD® AbSeq Ab-Oligos, using an 8-Channel Screw Cap Tube Capper and multichannel 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. 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 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 | 1 sample (µL) | 1 sample + 30% overage (µL) | 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) | 200 – (2.0 × *N*) | 260 – (2.6 × *N*) | 520 – (5.2 × *N*) |
| **Total** | **200.0** | **260** | **520** |
| (*N* = number of antibodies) | | | |

**Examples of different pools of BD® AbSeq Ab-Oligos are described below.**

| Component | 1 sample (µL) | 1 sample + 30% overage (µL) | 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) | 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) | 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) | 20 | 26 | 52 |
| **Total** | **200** | **260** | **520** |

**4** Pipet-mix the BD® 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, or at an appropriate speed to pellet the cells.

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

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) | 20.0 | 24.0 |
| Human BD Fc Block™ or Mouse BD Fc Block™ | 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** Discard 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 (15–25 °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 Discard 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 BD® 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 washing is 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. Users 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, or at an appropriate speed to pellet the cells.
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 residual supernatant from tube rim.
4. Add 3 mL BD® Stain Buffer to each tube and resuspend by pipet-mixing for the first wash.
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 the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove 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 cold Sample Buffer from the BD Rhapsody™ Enhanced Cartridge Reagent Kit. 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 recommended buffer and volume according to manufacturer.
9. Place the 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 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.<br>• Label BD® AbSeq and fluorescent antibody together to reduce cell loss during multiple washing steps, and proceed to cartridge loading right after sorting. |

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