BD Rhapsody™ System

Single-Cell Labeling with BD® AbSeq Ab-Oligos for Intracellular Proteomic Profiling

Protocol

23-24897(01)
2025-07

Regulatory information

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

History

Revision	Date	Change made
23-24897(01)	2025-07	Initial release.

Contents

Introduction

This protocol details how to use intracellular BD® AbSeq (IC-AbSeq) antibodies for profiling cells with the BD Rhapsody™ system without the need for mRNA. Each BD® IC-AbSeq antibody is conjugated to an antibody-specific oligonucleotide barcode to allow for single-cell profiling.

This protocol should be used alongside *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-24262) or *BD Rhapsody™ System Single-Cell Labeling with the BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-21339) to support single-cell labeling with surface BD® AbSeq Ab-Oligos without and with sample multiplexing, respectively. The following protocols are available for surface use with high-plex experiments:

- *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex) Protocol* (doc ID: 23-22314) for use instead of doc ID: 23-24262.
- *BD Rhapsody™ System Single-Cell Labeling with the BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos Protocol* (doc ID: 23-22354) for use instead of doc ID: 23-21339.

Immediately following this protocol, users will proceed to protocol *Single-Cell Capture and cDNA Synthesis with the BD Rhapsody™ Single-Cell Analysis System* (doc ID: 23-22951) or *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (doc ID: 23-24252). For library-preparation instructions, follow the guidance in *BD® AbSeq Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID: 23-24227) or *BD® AbSeq and Sample Tag Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID: 23-24228).

Symbols

The following symbols are used in this guide.

Symbol	Description
	Important information for maintaining measurement accuracy or data integrity.
	Noteworthy information.
	Procedural stopping point.

Considerations

The BD® Antibody Oligo Panel composition: The panel must include antibodies that can identify cell subtypes a user wants to explore.

Cell Viability: For optimal results, this protocol should be used with cells that have greater than 80% viability. If using cells with lower viability, the accuracy of cell calling may be impacted.

Required and recommended materials

Required materials

Store the reagents at the storage temperature specified on the label.

Material	Step	Supplier	Catalog no.
20,000–1 million cells	–	–	–
BD Pharmingen™ Stain Buffer (FBS)	Surface staining or sample multiplexing	BD	554656
BD® Hu Single-Cell Multiplexing Kit (SMK)—if sample multiplexing	Sample multiplexing	BD	633781
BD® OMICS-One Protein Panels	Surface AbSeq staining	BD	Various
BD® AbSeq Antibody=Oligos	Surface AbSeq staining	BD	Various
BD Pharmingen™ Transcription Factor Phospho (TFP) Buffer Set—see TFP Buffer Set components (page 6) for components	Fixation/permeabilization	BD	563239
BD Pharmingen™ Human BD Fc Block™	Surface and IC staining	BD	564220
Intracellular BD® AbSeq Ab-Oligo reagents	IC staining	BD	Various
BD® AbSeq Enhancer Kit Kit contains: • BD® AbSeq Enhancer 1, PN: 51-9022717 • BD® AbSeq Enhancer 2, PN: 51-9022718 • BD® AbSeq Enhancer 3, PN: 51-9022719	IC staining	BD	570750
BD Rhapsody™ Enhanced Cartridge Reagent Kit v3	Cartridge loading	BD	667052
BD Rhapsody™ Cartridge Kit or BD Rhapsody™ 8-Lane Cartridge	Cartridge loading	BD	633733 or 666262
BD Rhapsody™ cDNA Kit	cDNA synthesis	BD	633773
BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit	Library preparation	BD	663774
Vybrant™ DyeCycle™ Green Stain	BD Rhapsody™ cartridge loading	Thermo Fisher Scientific	V35004
INCYTO disposable hemocytometer	BD Rhapsody™ cartridge loading	INCYTO	CN DHC-N01-5
Nuclease-free water	Reconstituting the TFP Perm/Wash Buffer	Thermo Fisher Scientific	AM9937
100% Molecular Biology Grade Ethanol	BD Rhapsody™ cartridge loading	Major supplier	–

Material	Step	Supplier	Catalog no.
70% Ethanol or IPA	Sterilization	Major supplier	–
Proteinase K, Molecular Biology Grade	Cell lysis	New England Biolabs	P8107S
EDTA 0.5M	Exonuclease reaction	Thermo Fisher Scientific	J62786.AP

Where supplier is listed as "Major Supplier", products from any manufacturer or supplier are acceptable as long as they fit the material description.

TFP Buffer Set components

Material	Part number
0.75X TFP Diluent Buffer	51-9011462
4X TFP Fix/Perm Buffer	51-9011319
Perm Buffer III	51-9011461
5X TFP Perm/Wash Buffer	51-9011463

Recommended materials

Supplies	Supplier	Catalog no.
Single-channel pipettes (20, 200, 1000 µL)	Major supplier	–
Low-retention, filtered pipette tips for single channel pipettes	Major supplier	–
Serological pipette (15 mL)	Major supplier	–
Cell strainer cap	Corning	352235
Lint-free cloth (Kim-Wipes)	Major supplier	–
5-mL, 12×75 mm round-bottom polystyrene tubes.	Thermo Fisher Scientific	FB149563A
Eppendorf 5-mL DNA LoBind tubes	Eppendorf	0030122348
Eppendorf 1.5-mL Protein and DNA LoBind tubes	Eppendorf	022431021
Falcon® 15-mL conical centrifuge tubes	Major supplier	–

Required equipment

Equipment	Supplier	Catalog no.
Temperature controlled swinging-bucket centrifuge	Major supplier	–
37 °C incubator/water bath	Major supplier	–
Ice bucket	Major supplier	–
Sample vortexer	Major supplier	–
BD Rhapsody™ P1200M pipette	BD	–
BD Rhapsody™ P5000M pipette	BD	–
Thermoblock for 1.5-mL tubes, 37 °C, set to 1,200 rpm shaking	Major supplier	–
Thermoblock for 1.5-mL tubes, 80 °C	Major supplier	–
Cell counter	Major supplier	–
BD Rhapsody™ Scanner and/or BD Rhapsody™ Express System or BD Rhapsody™ HT Xpress Package	BD	633701 and/or 633707 or 666625
Digital timer	Major supplier	–

Best practices

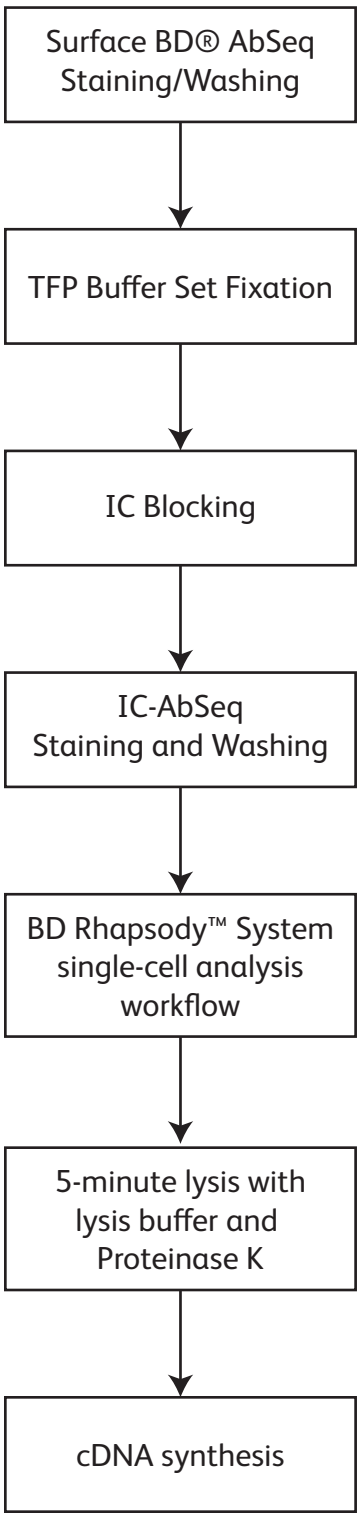
- Use only the materials specified in this protocol. Use of other materials could impact results.
- Store all reagents at the recommended storage temperatures with tightly sealed caps when not in use.
- Use low-retention filtered pipette tips.
- **Do not vortex** or **freeze** BD® AbSeq antibodies.
- Always use a swinging-bucket centrifuge for pelleting cells.
- It is important to keep cells and buffers on ice, especially after permeabilization.
- Keep centrifuge at 4 °C for all 800*g* centrifugation steps. Keep centrifuge lid closed between centrifugation steps to maintain temperature.
- For a complete list of materials for the BD Rhapsody™ System, refer to one of the following, as applicable to your system:
  - *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (docID: 23-21336)
  - *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide* (docID: 23-21332)
  - *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-free Workflow* (docID: 23-24256)
  - *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide* (docID: 23-24257)

The bead-retrieval magnet is lowered for 2 minutes during the bead-retrieval step of the cartridge workflow, which is longer than what might be written in other BD Rhapsody™ protocols.

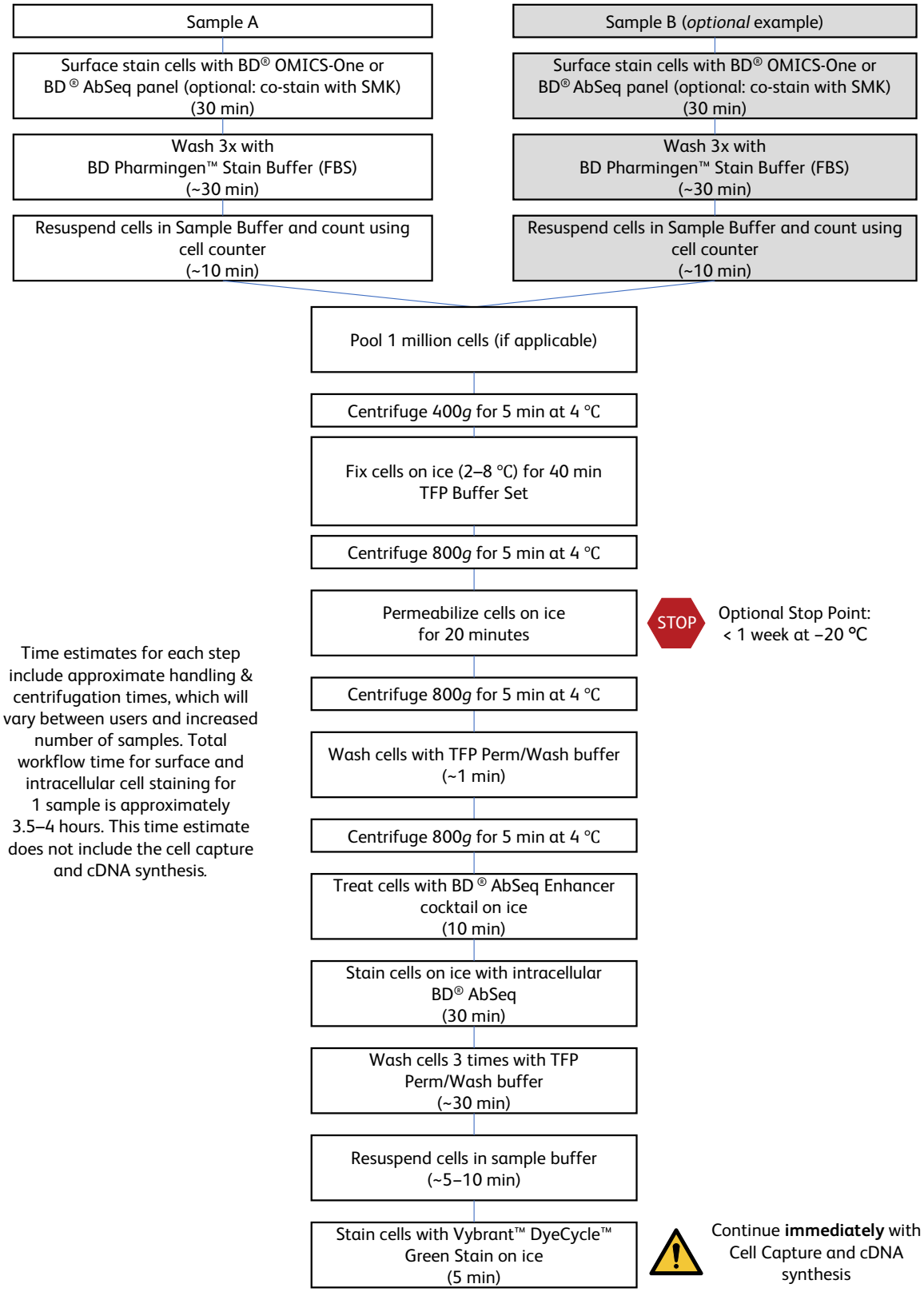
Additional documentation

- *BD Rhapsody™ System Single-Cell Labeling with the BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-21339)
- *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (41 plex to 100 plex) Protocol* (doc ID: 23-22354)
- *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-24262)
- *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (from 41 plex to 100 plex) Protocol* (doc ID: 23-22314)
- *BD Rhapsody™ System Preparing Single-Cell Suspensions Protocol* (doc ID: 23-24126)
- *BD Rhapsody™ Single Cell Capture and cDNA Synthesis with BD Rhapsody™ Single-Cell Analysis System Protocol* (doc ID: 23-22951)
- *BD Rhapsody™ Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Express Single-Cell Analysis System Protocol* (doc ID: 23-22952)
- *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol*, 23-24252
- *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol*(doc ID: 23-24253)
- *BD Rhapsody™ System BD® AbSeq Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID: 23-24227)
- *BD Rhapsody™ BD® AbSeq and Sample Tag Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID:23-24228)
- *Technical Data Sheet: Transcription Factor Phospho Buffer Set* (doc ID: 563239 Rev. 4)

Workflow overview



Time considerations



Before you begin

- Prepare a single-cell suspension. Refer to *BD Rhapsody™ System Preparing Single-Cell Suspensions Protocol* (doc ID: 23-24126).
- For detailed information on surface labeling with BD® AbSeq Ab-Oligos in a single tube, refer to one of the following protocols:
  - *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol*(doc ID: 23-24262)
  - *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex) Protocol* (doc ID: 23-22314)
  - *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol*(doc ID: 23-21339)
  - *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (41 plex to 100 plex) Protocol*(doc ID: 23-22354)
- For detailed information on surface labeling with BD® OMICS-One Protein Panels, refer to the Technical Data Sheet of each panel.
- If working with stimulated or cultured cells, it is recommended to strain cells through a 35 µm cell strainer cap (Corning catalog no. 352235) before beginning BD® AbSeq staining.
- The BD® Single-Cell Multiplexing Kit can be co-stained with either surface AbSeq or IC-AbSeq. Plan to include it according to the experimental design.
- Prepare the TFP Fix/Perm Buffer fresh before fixation. Refer to *Technical Data Sheet: Transcription Factor Phospho Buffer Set* (doc ID: 563239 Rev. 4) for more information.
- Keep Perm Buffer III stored at –20 °C and take it out immediately before the permeabilization step.

Safety information

Perm Buffer III contains 87.68% Methanol (CAS number 67-56-1). This reagent is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and Regulation (EC) No 1272/2008. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Danger
	H225: Highly flammable liquid and vapor. H301+H311+H331: Toxic if swallowed, in contact with skin or if inhaled. H370: Causes damage to organs.
Prevention	P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P240: Ground and bond container and receiving equipment. P241: Use explosion-proof [electrical/ventilating/lighting] equipment. P242: Use non-sparking tools. P243: Take action to prevent static discharges. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P301+P310: IF SWALLOWED: Immediately call a POISON CENTER/doctor. P330: Rinse mouth. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P363: Wash contaminated clothing before reuse. P312: Call a POISON CENTER or doctor/physician if you feel unwell. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P311: Call a POISON CENTER or doctor/physician. P307+P311: IF exposed or concerned: Call a POISON CENTER/doctor. P370+P378: In case of fire: Use dry sand, dry chemical or alcohol resistant foam for extinction.
Storage	P403+P233: Store in a well-ventilated place. Keep container tightly closed. P405: Store locked up.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

For additional safety information, refer to the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (doc ID: 23-21336) or the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide* (doc ID: 23-21332).

Preparation

Surface staining of cells

Perform the experiment using either of the following BD Rhapsody™ System Single-Cell Labeling protocols:

- *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-24262) or *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex) Protocol*(doc ID: 23-22314)

  STOP after the section "Washing labeled cells" and follow this protocol from Step 1: Fixation and permeabilization of the cells (page 13) and subsequent steps.

- *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex) Protocol* (doc ID: 23-21339) or *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos Protocol*(doc ID: 23-22354)

  STOP after the section "Washing the labeled cells" and follow this protocol from Step 1: Fixation and permeabilization of the cells (page 13) and subsequent steps. It is also possible to co-stain Human SMK with the IC Stain Mix (see instructions in Step 2: Labeling cells with BD® IC-AbSeq Ab-Oligos (page 15) and the example in Example of 10-plex BD® IC-AbSeq + Human SMK Labeling Master Mix (page 16)).

Buffer preparation

- Prepare the 1X TFP Perm/Wash Buffer from the 5X TFP Perm/Wash Buffer solution by diluting with filtered, nuclease-free water (e.g., use 10 mL of 5X TFP Perm/Wash Buffer stock and mix with 40 mL of nuclease-free water).
- Prepare the 1X TFP Fix/Perm Buffer 10–15 minutes prior to use. Mix 4X TFP Fix/Perm Buffer stock with 0.75X TFP Diluent Buffer to make 1X TFP Fix/Perm Buffer (e.g., use 10 mL of 4X TFP Fix/Perm Buffer stock and mix with 30 mL of 0.75X TFP Diluent Buffer).
- Thaw reagents (except for Proteinase K) at room temperature (15–25 °C) and place back on ice. Keep Proteinase K at –25 °C to –15 °C. All components should be properly capped or sealed and stored when not in use.
- Prepare the buffer mixes on the same day you perform the intracellular staining and single-cell capture.

Procedure

This procedure comprises the following tasks:

Step 1: Fixation and permeabilization of the cells

Surface AbSeq-stained cells should be washed three times with BD stain buffer with residual supernatant carefully removed. If cells have been tagged with Sample Tags and will be stained with the same IC markers, we

recommend resuspending in sample buffer, and pooling cells at this point, aiming for a total of 250,000–1 million cells. The cell pool should be centrifuged again at 400*g* for 5 minutes and supernatant discarded.

The cell pellet is then ready for fixation, permeabilization, and intracellular staining, as described in the following substeps:

1. Gently tap the bottom of the tube on the work bench to break the cell pellet, and add 5 mL of 1X TFP Fix/Perm Buffer to the cell pellet. Gently pipette mix the samples 10 times to avoid bubble formation.
2. Transfer cells from 5-mL round-bottom polystyrene tube to a 15-mL Falcon tube. Avoid using DNA LoBind tubes for fixation and permeabilization steps.
3. Incubate the cells in 1X TFP Fix/Perm Buffer at 2–8 °C for 40 minutes.
4. Add 5 mL of 1X TFP Perm/Wash Buffer.
5. Using a swinging-bucket centrifuge, pellet cells at 800*g* for 5 minutes at 4 °C. Carefully remove and discard the supernatant without disturbing the cell pellet.
6. Wash cells once with 10 mL 1X TFP Perm/Wash Buffer.
7. Centrifuge cells at 800g for 5 minutes at 4 °C. Aspirate all residual supernatant volume away from the pelleted cells.
8. Gently tap the tube on the work bench at least 10 times to break the cell pellet.
9. Change vortex to slowest speed and use continual vortexing mode (do not use touch mode).
10. While slowly vortexing the cells, add drops equaling 1 mL of ice-cold Perm Buffer III.

    a. Vortexing is critical to prevent cell multiplets.
    b. Take Perm Buffer III from a –20 °C freezer immediately before use.
    c. Cell pellets become translucent during this substep.

11. Permeabilize the cells on ice for 20 minutes.

    During this incubation, if not stopping, we recommend preparing the BD® IC-AbSeq Labeling Master Mix following BD® IC-AbSeq Labeling Master Mix (page 15) in the section Labeling cells with BD® IC-AbSeq Ab-Oligos (page 15).

    STOP **OPTIONAL STOPPING POINT:** After cells have been permeabilized, they can be stored at –20 °C in Perm Buffer III for up to one week.

12. Using a swinging-bucket centrifuge, pellet the cells at 800*g* for 5 minutes at 4 °C.
13. Carefully remove and discard as much Perm Buffer III as possible with a P1000 pipette without disturbing the cell pellet.
14. Gently tap the tube to break the cell pellet and add 1 mL of 1X TFP Perm/Wash Buffer, then pipet-mix 5–10 times.
15. Centrifuge at 800*g* for 5 minutes at 4 °C. Continue this protocol from Labeling cells with BD® IC-AbSeq Ab-Oligos (page 15).

Step 2: Labeling cells with BD® IC-AbSeq Ab-Oligos

Before pooling intracellular antibodies for staining, we recommend centrifuging them at 400*g* for 30 seconds prior to uncapping and placing on ice.

1. In a new 1.5-mL LoBind tube, pipet the reagents listed in BD® IC-AbSeq Stain Buffer Mix (Protein only) (page 15). The table makes enough BD® IC-AbSeq Stain Buffer Mix (Protein only) for one sample only.

Table 1: BD® IC-AbSeq Stain Buffer Mix (Protein only)

BD® IC-AbSeq Stain Buffer Component	Volume (µL)
1X TFP Perm/Wash Buffer	84.0
BD® AbSeq Signal Enhancer 1	12.0[a]
BD® AbSeq Signal Enhancer 2	12.0[a]
BD® AbSeq Signal Enhancer 3	12.0[a]
Total	**120.0**

a. Certain cell type and target combinations may require the use of increased BD® AbSeq Enhancer concentrations to achieve better IC-AbSeq signal-to-noise ratio. We recommend optimizing for your sample type.

It is important to retain the remaining 50 µL of the IC-AbSeq Stain Buffer mix (Protein only) and keep it on ice until required.

2. Prepare 2X BD® IC-AbSeq Labeling Master Mix, by pipetting the following reagents into a new 1.5-mL DNA LoBind tube.

Table 2: BD® IC-AbSeq Labeling Master Mix

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
Per BD® IC-AbSeq Ab-Oligo	2.0	2.6	5.2
BD® IC-AbSeq Stain Buffer Mix (Protein only) (N = number of antibodies)	50.0 – (2.0 × N)	65.0 – (2.6 × N)	130.0 – (5.2 × N)
Total	**50.0**	**65.0**	**130.0**

Table 3: Example of 10-plex BD® IC-AbSeq Labeling Master Mix

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
Per BD® IC-AbSeq Ab-Oligo	2.0 (20.0 total)	2.6 (26.0 total)	5.2 (52.0 total)
BD® IC-AbSeq Stain Buffer Mix (Protein only)	30.0	39.0	78.0
Total	**50.0**	**65.0**	**130.0**

Table 4: Example of 10-plex BD® IC-AbSeq + Human SMK Labeling Master Mix

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% Overage (µL)
Per BD® IC-AbSeq Ab-Oligo	2.0 (20.0 total)	2.6 (26.0 total)	5.2 (52.0 total)
BD®IC-AbSeq Stain Buffer Mix (Protein only)	10.0	19.0	38.0
Total	**30.0**	**45.0**	**90.0**

To each Sample Tag tube containing 20 µL of Sample Tag, add 30 µL of BD® IC-AbSeq Labeling Master Mix.

We recommend:

- Creating freshly pooled antibodies before each experiment.
- Creating pools with 30% overage to ensure adequate volumes for pipetting. The reagents are viscous and form bubbles easily. Avoid creating bubbles by pipetting slowly.
- For high-plex panels, using an 8-Channel Screw Cap Tube Capper and multi-channel pipette to pipet BD® IC-AbSeq Ab-Oligos into 8-tube strips. Centrifuge tube strip and pool 2X BD® IC-AbSeq Ab-Oligos into a 1.5-mL DNA LoBind tube.

3. Pipet the BD® IC-AbSeq Labeling Master Mix and place on ice.
4. Carefully remove and appropriately discard the supernatant from the sample tube from step 1.15 and gently tap to break the cell pellet.
5. Resuspend cells in 50 µL of BD® IC-AbSeq Stain Buffer mix (Protein only) and incubate on ice for 10 minutes. This incubation step is critical to allow the entry of AbSeq Enhancers into cells.
6. After a 10-minute incubation, add 50 µL of BD® IC-AbSeq Labeling Master Mix to the tube containing 50 µL of the cell suspension to make a final volume of 100 µL cell staining mix. Pipet-mix well and incubate on ice for 30 minutes.

Step 3: Washing labeled cells

1. Add 2 mL of 1X TFP Perm/Wash Buffer to the labeled cells and pipet mix.
2. Using a swinging-bucket centrifuge, pellet cells at 800*g* for 5 minutes at 4 °C.
3. Invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wipe to remove residual supernatant from the tube.
4. Repeat substeps 1–3 for a total of three washes.

Step 4: Preparation for single-cell capture and cDNA synthesis

1. Resuspend the pellet in 620 µL of **cold sample buffer** and transfer to a new 1.5-mL DNA LoBind tube.

   For low-cell concentrations (below 500,000 cells starting amount), we recommend to resuspend cells in 310 µL cold sample buffer and halve the volume of Vybrant™ DyeCycle™ Green Stain to 1.6 µL in the next substep.

2. Add 3.1 µL of Vybrant™ DyeCycle™ Green Stain to the cells (1.6 µL for 310 µL cell suspension) and gently pipet-mix 8–10 times. Incubate on ice for 5 minutes while protecting from light.
3. Filter cells through a Falcon tube with Cell Strainer Cap.
4. Count cells immediately using the BD Rhapsody™ Scanner by gently pipetting 10 µL of cells into an INCYTO disposable hemocytometer.

   Since these are fixed cells, cell viability is not applicable.

5. Keep remaining cells on ice until single-cell capture steps have completed.
   - For use with BD Rhapsody™ HT Xpress 8-lane cartridge workflow, skip to the section Single-cell capture and cDNA synthesis using HT Xpress protocol (page 17).
   - See Protocols to Follow for Cell Capture and cDNA Synthesis Step (page 17) for information on the necessary protocol for cell capture and cDNA synthesis.

Table 5: Protocols to Follow for Cell Capture and cDNA Synthesis Step

Cartridge Type	Protocol	Step
BD Rhapsody™ 8-Lane Cartridge	With BD Rhapsody™ Scanner: *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol*, 23-24252 or Without BD Rhapsody™ Scanner: *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol*, 23-24253	Priming and loading BD Rhapsody™ 8-Lane Cartridge

Step 5: Single-cell capture and cDNA synthesis using HT Xpress protocol

For the following section, proceed using the appropriate Single-Cell Capture and cDNA synthesis protocol listed in Protocols to Follow for Cell Capture and cDNA Synthesis Step (page 17) to find the correct protocol. However, be sure to include the following modifications outlined in the following substeps.

1. Prepare a modified lysis buffer with Proteinase K and perform lysis and retrieval substeps as follows:
   a. Immediately before the following lysis substep, prepare the modified lysis buffer with Proteinase K using Modified Lysis Buffer with Proteinase K for HT Xpress Cartridge (page 18). Keep on ice.
   b. Lyse the cells for 5 minutes (instead of 2 minutes) at room temperature (15–25 °C).
   c. During retrieval, use lysis buffer with DTT (no Proteinase K) and increase the time with the retrieval magnet set in the down position from 30 seconds to *2 minutes*.

      It is critical to keep the magnet in retrieval position for 2 minutes to maximize bead recovery.

   d. To deactivate Exonuclease reaction, add 4 µL of 0.5M EDTA to the 200 µL reaction mixture and pipet-mix.

Table 6: Modified Lysis Buffer with Proteinase K for HT Xpress Cartridge

Cartridge Type	Vol. of Lysis Buffer with DTT (µL)	Vol. of Proteinase K (µL)	Step
BD Rhapsody™ 8-Lane Cartridge (1 lane)	250	13	Lysis
BD Rhapsody™ 8-Lane Cartridge (8 lanes)	2,000	100	Lysis

2. Use *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (doc ID: 23-24252) for instructions on cDNA synthesis. After cDNA synthesis, exonuclease-treated beads can be stored at 2–8 °C for up to 3 months before proceeding to library preparation using the BD Rhapsody™ AbSeq-only protocols:
   - *BD Rhapsody™ System BD® AbSeq Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID: 23-24227)
   - *BD Rhapsody™ System BD® AbSeq and Sample Tag Library Preparation Protocol (for AbSeq-based cell calling)* (doc ID: 23-24228)

This protocol is not compatible with BD Rhapsody™ nucleic acid detection assays, such as WTA, Targeted mRNA, TCR/BCR profiling, or scATAC-Seq. The BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit is used for the preparation of AbSeq and SMK libraries only.

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