

 BD Rhapsody™ System
Single-Cell Labeling with BD® AbSeq
Ab-Oligos for Intracellular CITE-seq
Protocol

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Regulatory information

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

History

Revision	Date	Change made
23-25005(01)	2025-10	Initial release.

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Introduction

This protocol details how to stain for intracellular antigens using intracellular BD® AbSeq (IC-AbSeq) antibodies for profiling with the BD Rhapsody™ system. Each BD® IC-AbSeq antibody is conjugated to an antibody-specific oligonucleotide barcode for profiling alongside surface protein and mRNA expression. Note that 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 BD® AbSeq Ab-Oligos without and with sample multiplexing, respectively. This protocol has been tested for use with up to 40 surface markers. The following protocols are available for use with high-plex experiments (however, additional optimization may be required):

- *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 the BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos Protocol*, (doc ID: 23-22354)

See [Procedure \(page 16\)](#) for notes on how to use this protocol in conjunction.

Immediately following this protocol, users will proceed to a protocol for Single-Cell Capture and cDNA Synthesis using one of the protocols listed in [Protocols to Follow for Cell Capture and cDNA Synthesis Step \(page 21\)](#).



The BD® IC-AbSeq protocol is not compatible with BD Rhapsody™ Targeted Assay or TCR BCR Assay Kits.

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.

Required and recommended materials

Intracellular BD Rhapsody™ AbSeq Buffer Kit contents (catalog no. 570742)

Material	Part number
Perm Buffer	51-9022686
Base Buffer	51-9022685
Proteinase K, Molecular Biology Grade	51-9022689
0.1M DTT, Molecular Biology Grade	51-9022688
Nuclease-free water	51-9022687

For best results, use required and recommended materials and equipment from the indicated supplier.

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® Single-Cell Multiplexing Kit if sample multiplexing	Human sample multiplexing kit	BD	633781
BD OMICS-One™ Immune Profiler Protein Panel	Surface AbSeq staining	BD	571970
BD OMICS-One™ B-cell Protein Panel	Surface AbSeq staining	BD	572179
BD OMICS-One™ T-cell Protein Panel	Surface AbSeq staining	BD	572178
BD OMICS-One™ Tumor Protein Panel	Surface AbSeq staining	BD	572310
BD OMICS-One™ NK-Cell Protein Panel	Surface AbSeq staining	BD	572434
BD OMICS-One™ APC/Myeloid-Cell Protein Panel	Surface AbSeq staining	BD	572435
Intracellular BD® AbSeq Panel	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

Material	Step	Supplier	Catalog no.
BD® RNase Inhibitor	Cell hydration, IC staining, IC washing, cell suspension, cell capture	BD	570751
BD OMICS-Guard™ Sample Preservation Buffer Kit	Fixation	BD	570908
BD Rhapsody™ Enhanced Cartridge Reagent Kit	Cartridge loading	BD	664887
BD Rhapsody™ Cartridge Kit or BD Rhapsody™ 8-Lane Cartridge	Cartridge loading	BD	633733 or 666262
BD Rhapsody™ cDNA Kit	cDNA synthesis	BD	633773
Vybrant™ DyeCycle™ Green Stain	BD Rhapsody™ cartridge loading	Thermo Fisher Scientific	V35004
INCYTO disposable hemocytometer	BD Rhapsody™ cartridge loading	INCYTO	CN DHC-N01-5
100% Molecular Biology Grade Ethanol	BD Rhapsody™ cartridge loading	Major supplier	–
70% Ethanol or IPA	Sterilization	Major supplier	–

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

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
BD Pharmingen™ Human BD Fc Block™	BD	564220
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

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	–

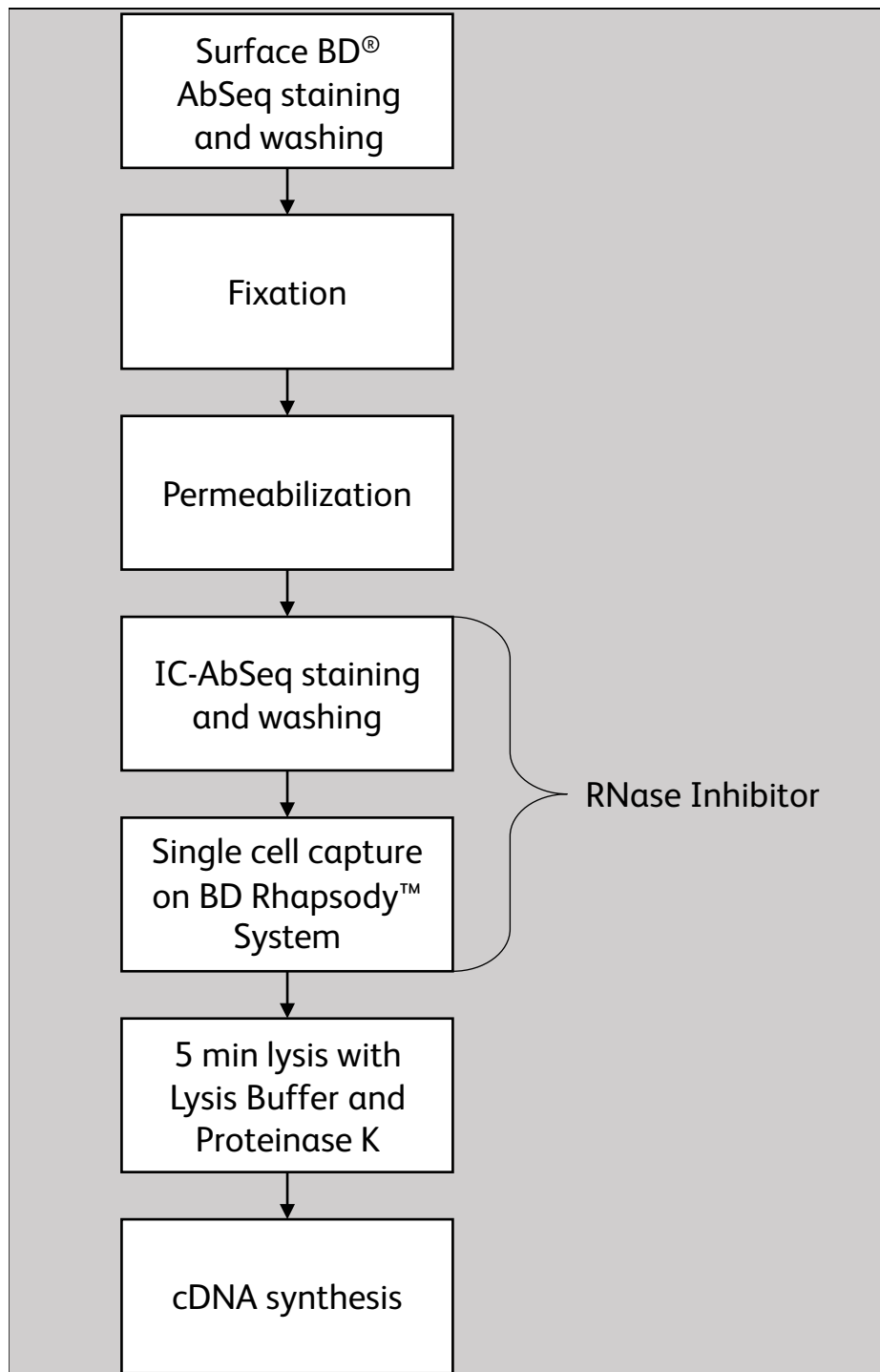
Best practices

- 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 800g 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*, (doc ID: 23-21336)
 - *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide*, (doc ID: 23-21332)
 - *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-free Workflow*, (doc ID: 23-24256)
 - *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide*, (doc ID: 23-24257)
- The transient phosphorylation status of certain markers can result in dephosphorylation during surface AbSeq staining. If it is a concern, proceed immediately with 5 min fixation using BD OMICS-Guard™ Sample Preservation Buffer and immediately permeabilize cells prior to surface staining. Once cells are permeabilized and treated with Cell Hydration Buffer, co-stain using surface and intracellular BD® AbSeq Ab-Oligo reagents (with BD Pharmingen™ Human BD Fc Block™ if required). For a list of compatible surface antibody clones, refer to *Antibodies to Human Cell-Surface Markers Tested for BD Phosflow™ Protocols*, (doc ID: 23-11080). Do not stop and store cells at the OMICS-Guard™ step. If you need further guidance, contact your local Field Application Specialist (FAS).

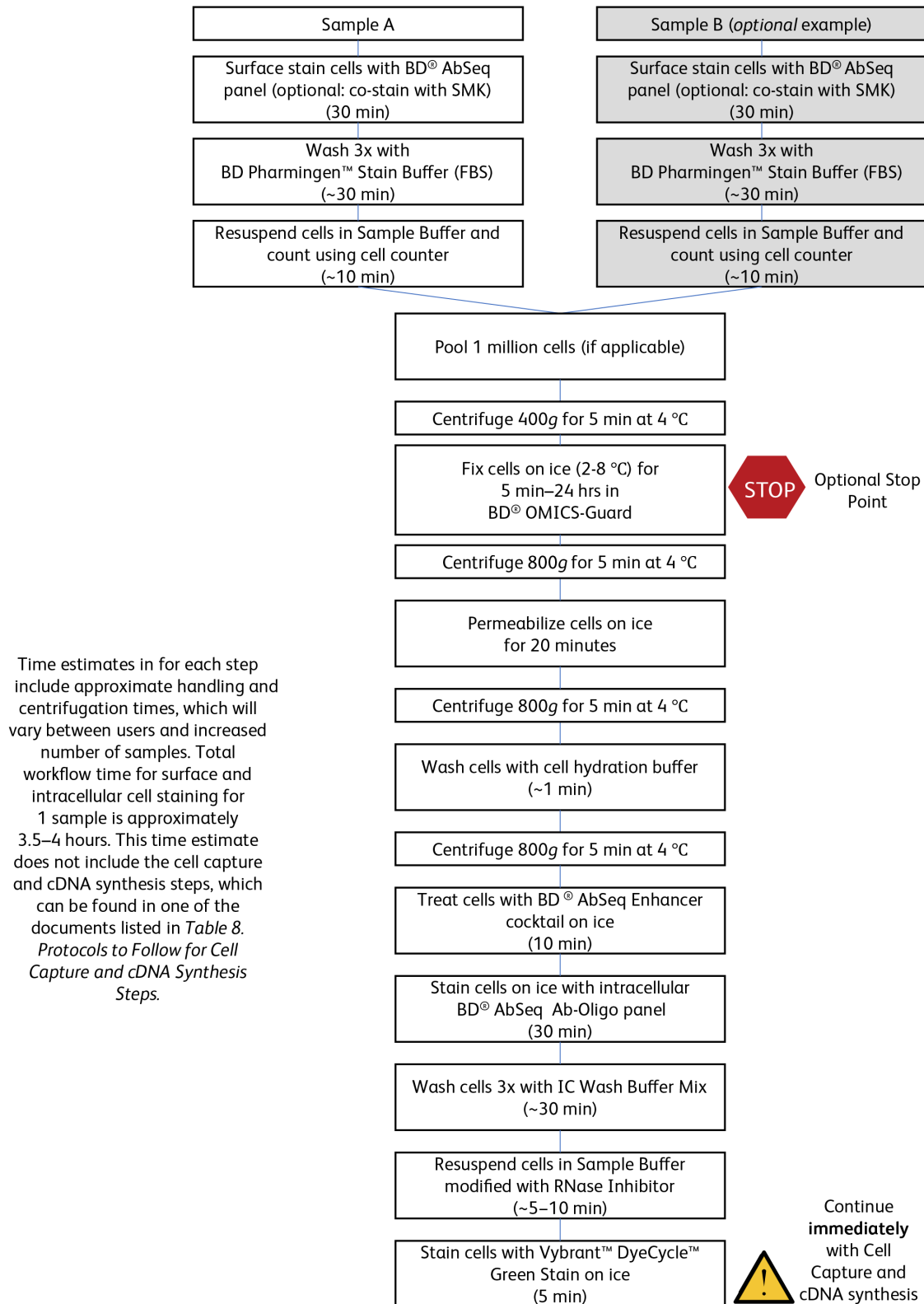
Additional documentation

- *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* (doc ID: 23-24252)
- *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol* (doc ID: 23-24253)
- *BD Rhapsody™ BD OMICS-One™ WTA Next and BD® AbSeq Library Preparation Protocol* (doc ID: 23-24492)
- *BD Rhapsody™ BD OMICS-One™ WTA Next, BD® AbSeq, and Sample Tag Library Preparation Protocol* (doc ID: 23-24494)
- *BD OMICS-One™ Immune Profiler Protein Panel* (doc ID: 571970 Rev. 01)
- *BD OMICS-One™ T-Cell Protein Panel* (doc ID: 572178 Rev. 01)
- *BD OMICS-One™ B-Cell Protein Panel* (doc ID: 572179 Rev. 01)
- *BD OMICS-One™ Adaptive Protein Panel* (doc ID: 572241 Rev. 01)
- *BD OMICS-One™ Immuno-Oncology Protein Panel* (doc ID: 572316 Rev. 01)
- *BD OMICS-One™ Tumor Protein Panel* (doc ID: 572310 Rev. 02)

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).
- 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.
- For detailed information on surface labeling with BD® AbSeq Ab-Oligos, or co-labeling cells with Sample Tag and BD® AbSeq Ab-Oligos in a single tube, refer to [Additional documentation \(page 9\)](#)
- 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.
- Create modified sample buffer by adding RNase inhibitor (25 µL/mL) to the sample buffer from the BD Rhapsody™ Enhanced Cartridge Reagent Kit (catalog no. 664887). Users need 4 mL of modified sample buffer per single-lane cartridge or 3 mL of modified sample buffer per lane for 8-lane cartridge. See either [Modified Sample Buffer with RNase Inhibitor for Single-Lane Cartridge \(page 21\)](#) or [Modified Sample Buffer with RNase Inhibitor for HT Xpress Cartridge \(page 23\)](#) for the correct volume of modified sample buffer to prepare.



The concentration for BD® RNase Inhibitor is optimized for use with human peripheral blood mononuclear cells (PBMCs). If other cell types are used, we recommend that users test for optimal concentration. Use only BD® or New England Biolabs RNase Inhibitor (catalog no. M0314L). Use of other RNase inhibitors will impact experiment.

Buffer mix tables for intracellular staining

All buffer mix tables make enough buffer for one intracellular staining test. Thaw reagents (except for BD® RNase Inhibitor and Proteinase K) at room temperature (15–25 °C) and place back on ice. Keep BD® RNase Inhibitor and 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. We recommend adding RNase inhibitor just before use.

1. In a new 15-mL nuclease-free conical tube, pipet the reagents in [IC Wash Buffer Mix \(page 13\)](#), and place back on ice.

Table 1: IC Wash Buffer Mix

IC Wash Buffer Component	Part number/Catalog no.	Amount (μL)
Base Buffer	51-9022685	3,000 (3 mL)
BD® RNase Inhibitor	570751	70
Nuclease-free water	51-9022687	4,000 (4 mL)
Total	–	7,070 (7.070 mL)

2. In a new 1.5-mL LoBind tube, pipet the reagents in [Cell Hydration Buffer Mix \(page 13\)](#), and place back on ice.

Table 2: Cell Hydration Buffer Mix

Cell Hydration Buffer Component	Part number/Catalog no.	Amount (μL)
Base Buffer	51-9022685	465
BD® RNase Inhibitor	570751	30
Nuclease-free water	51-9022687	635
Total	–	1,130

3. In a new 1.5-mL LoBind tube, pipet the reagents in [BD® IC-AbSeq Stain Buffer Mix \(page 14\)](#), and place back on ice.

Table 3: BD® IC-AbSeq Stain Buffer Mix

BD® IC-AbSeq Stain Buffer Component	Part number/Catalog no.	Volume (μL)
Base Buffer	51-9022685	48
BD® AbSeq Enhancer 1	51-9022717	12 ^a
BD® AbSeq Enhancer 2	51-9022718	12 ^a
BD® AbSeq Enhancer 3	51-9022719	12 ^a
BD® RNase Inhibitor	570751	3.8
0.1M DTT	51-9022688	1.2
Nuclease-free water	51-9022687	31 ^a
Total	–	120^a

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. We recommend optimizing for your sample type.

When increasing the BD® AbSeq Enhancer volume, decrease the amount of water. For example, if increasing the concentration of Enhancer by 1.5X, add 18 μL of each Enhancer and 13 μL of nuclease-free water. If increasing the concentration by 2X, add 24 μL of each Enhancer and 0 μL of nuclease-free water. The final volume may increase up to 125 μL.

Safety information

The Perm Buffer 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).

Procedure

This procedure comprises the following tasks:

- [Fixation and permeabilization of the cells \(page 16\)](#)
- [Labeling cells with BD® IC-AbSeq Ab-Oligos \(page 17\)](#)
- [Washing labeled cells \(page 19\)](#)
- [Single-cell capture and cDNA synthesis \(page 20\)](#)
- [HT Xpress protocol \(page 22\)](#)



Do not prepare buffers from Buffer Mix tables ([IC Wash Buffer Mix \(page 13\)](#), [Cell Hydration Buffer Mix \(page 13\)](#), and [BD® IC-AbSeq Stain Buffer Mix \(page 14\)](#)) until the day cartridge loading will be performed.

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” in step 7 and follow this protocol from [Fixation and permeabilization of the cells \(page 16\)](#) and subsequent steps.

OR

- *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” on step 7 and follow this protocol from [Fixation and permeabilization of the cells \(page 16\)](#) and subsequent steps. It is also possible to co-stain Human SMK with the IC Stain Mix (see instructions following [Labeling cells with BD® IC-AbSeq Ab-Oligos \(page 17\)](#) and the example in [Example of 10-plex BD® IC-AbSeq + Human SMK Labeling Master Mix \(page 18\)](#)).

Ensure that the intended total cell loading is between 20,000 to 40,000 single cells per cartridge lane for this protocol. Cell loading below or above this recommended range may not be suitable for current protocol configuration. Then proceed as described in the following procedure.

Fixation and permeabilization of the cells

Surface-stained cells should be washed three times with BD stain buffer with residual supernatant carefully removed. If cells have been sample tagged using the BD® Human Single-Cell Multiplexing Kit (SMK), we recommend resuspending in Sample Buffer (without BD® RNase Inhibitor) and pooling cells at this point, aiming for 250,000–1 million cells. The cell pool should be centrifuged again at 400g for 5 minutes and supernatant discarded. It is also possible to obtain cell counts and pool the cells while in BD OMICS-Guard™ Sample Preservation Buffer and pool sample tagged cells before step 4.

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

1. Gently tap the bottom of the tube on the work bench to break the cell pellet, and add 1 mL of BD OMICS-Guard™ Sample Preservation Buffer to the cell pellet. Gently pipet-mix the samples 10 times to avoid bubble formation.

2. If cells are not already in a 5-mL 12×75 mm round-bottom polystyrene tube, transfer them at this point. Avoid using DNA LoBind tubes for fixation and permeabilization steps.
3. Incubate the cells in BD OMICS-Guard™ Sample Preservation Buffer on ice for 5 minutes.



OPTIONAL: It is possible to store cells in the BD OMICS-Guard™ Sample Preservation Buffer for up to 24 hours and resume step 4 the following day.



BD OMICS-Guard™ Sample Preservation Buffer has been tested for 24-hour storage at 4 °C using Human PBMCs. If using cells other than PBMCs, users are advised to test their sample type before storing cells longer than 40 minutes.

4. Using a swinging-bucket centrifuge, pellet cells at 800g for 5 minutes at 4 °C. Carefully decant supernatant into biohazardous waste by inverting tube. Keep the tube inverted and gently blot on a lint-free wipe without disturbing the cell pellet.
5. Gently tap the tube on the work bench at least 10 times to break the cell pellet.
6. Change vortex to slowest speed and continual vortexing mode (do not use touch mode).



Wear appropriate personal protective equipment, including eye protection, laboratory coat, and protective gloves to avoid methanol exposure.

7. While slowly vortexing the cells, add drops equaling 1 mL of ice-cold Perm Buffer.



Vortexing is critical to prevent cell multiplets.

8. Permeabilize the cells on ice for 20 minutes.



During this incubation, we recommend preparing the BD® IC-AbSeq Labeling Master Mix following [BD® IC-AbSeq Labeling Master Mix \(page 18\)](#), [Example of 10-plex BD® IC-AbSeq Labeling Master Mix \(page 18\)](#) or [BD® IC-AbSeq Labeling Master Mix + Human SMK Labeling Master Mix \(page 18\)](#), in the section [Labeling cells with BD® IC-AbSeq Ab-Oligos \(page 17\)](#)

9. Using a swinging-bucket centrifuge, pellet the cells at 800g for 5 minutes at 4 °C.
10. Carefully remove and discard as much Perm Buffer as possible with a P1000 pipette without disturbing the cell pellet.
11. Add BD® RNase Inhibitor to Cell Hydration Buffer Mix, if not previously added. See [Cell Hydration Buffer Mix \(page 13\)](#) in section [Buffer mix tables for intracellular staining \(page 13\)](#) Gently vortex mix, briefly centrifuge, and place back on ice.
12. Gently tap the tube to break the cell pellet and add 1 mL of Cell Hydration Buffer Mix, then gently pipet-mix 5–10 times to avoid bubble formation.
13. Centrifuge at 800g for 5 minutes at 4 °C. Continue this protocol from [Labeling cells with BD® IC-AbSeq Ab-Oligos \(page 17\)](#).

Labeling cells with BD® IC-AbSeq Ab-Oligos



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

14. Add BD[®] RNase Inhibitor to BD[®] IC-AbSeq Stain Buffer Mix, if not previously added. See to [BD[®] IC-AbSeq Stain Buffer Mix \(page 14\)](#) in section [Buffer mix tables for intracellular staining \(page 13\)](#) Gently vortex mix, briefly centrifuge, and place back on ice.
15. Prepare BD[®] IC-AbSeq Labeling Master Mix, by pipetting the following reagents into a new 1.5-mL DNA LoBind tube. All components should be properly capped/sealed and stored when not in use.



It is important to retain the remaining 50 µL of the BD[®] IC-AbSeq Stain Buffer for the blocking step.

Table 4: 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 (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 5: BD[®] IC-AbSeq Labeling Master Mix + 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	2.6	5.2
BD [®] IC-AbSeq Stain Buffer Mix (N = number of antibodies)	30.0 – (2.0 × N)	45.0 – (2.6 × N)	90.0 – (5.2 × N)
Total	30.0	45.0	90.0

Table 6: 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	30.0	39.0	78.0
Total	50.0	65.0	130.0

Table 7: 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	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 BD® IC-AbSeq Ab-Oligos into a 1.5-mL DNA LoBind tube.

16. Pipet the BD® IC-AbSeq Labeling Master Mix and place on ice.
17. Carefully decant supernatant into biohazardous waste by inverting tube from [step 13](#). Keep the tube inverted and blot on a lint-free wipe without disturbing the cell pellet.
18. Gently tap the tube on the work bench at least 10 times to break the cell pellet. Resuspend cells with the 50 µL of retained BD® IC-AbSeq Stain Buffer with BD® AbSeq Enhancer and incubate on ice for 10 minutes. This incubation step is critical to allow BD® AbSeq Enhancer into cells.
19. Add 50 µL of BD® IC-AbSeq Labeling Master Mix to the tube containing 50 µL of cell suspension plus BD® IC-AbSeq Stain Buffer with BD® AbSeq Enhancer, to make a final volume of 100 µL cell staining mix. Gently pipet-mix to avoid bubble formation and incubate on ice for 30 minutes. It is critical to not extend the incubation more than 30 minutes to limit RNase activity.



During this incubation, we recommend priming and treating BD Rhapsody™ Cartridge(s).



See [Protocols to Follow for Cell Capture and cDNA Synthesis Step \(page 21\)](#) following to find the correct protocol to follow for the BD Rhapsody™ System cartridge priming and treating steps.

Washing labeled cells

20. Add BD® RNase Inhibitor to IC Wash Buffer Mix, if not previously added. Refer to [IC Wash Buffer Mix \(page 13\)](#) in section [Buffer mix tables for intracellular staining \(page 13\)](#). Vortex mix and place back on ice.
21. Add 2 mL of IC Wash Buffer to the labeled cells and pipet mix.
22. Using a swinging-bucket centrifuge, pellet cells at 800g for 5 minutes at 4 °C.
23. 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.
24. Add 2 mL of IC Wash Buffer to cell pellets and resuspend by pipet mixing for the second wash.
25. Centrifuge with a swinging-bucket centrifuge at 800g for 5 minutes at 4 °C.
26. 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.
27. (Optional) Repeat [steps 24–26](#) for a total of 3 washes.

Single-cell capture and cDNA synthesis

Have you added RNase inhibitor to your sample buffer? (25 µL RNase Inhibitor/mL Sample Buffer. See [Introduction \(page 4\)](#) or [Modified Sample Buffer with RNase Inhibitor for HT Xpress Cartridge \(page 23\)](#) for details on how to prepare the Modified Sample Buffer.)

28. Visually inspect the Lysis Buffer (catalog no. 650000064) for any precipitation. If precipitation is present, incubate the Lysis Buffer at room temperature for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, place the Lysis Buffer on ice.
29. Open the tube while holding the DTT tube vertically. The solution is overlain with an inert/non-oxygencontaining gas, and a non-vertical tube will allow the inert gas to pour off. If not loading four cell cartridges (or lanes) at the same time, after opening the DTT tube once, seal and store the tube at –20 °C.
30. Resuspend the pellet in 620 µL of cold modified sample buffer.
 - For low-cell concentrations (below 500,000 cells starting amount), we recommend to resuspend cells in 310 µL cold modified sample buffer and halve the volume of Vybrant™ DyeCycle™ Green Stain to 1.6 µL in the next step. If unsure about concentration, use 310 µL.
31. 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.
32. Filter cells through a Falcon tube with Cell Strainer Cap.
33. Count cells immediately using the BD Rhapsody™ Scanner by gently pipetting 10 µL of cells into an INCYTO disposable hemocytometer.
34. Keep remaining cells on ice until single-cell capture steps have completed.



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

- For use with the BD Rhapsody™ Express single-lane cartridge workflow, proceed to [Single-lane cartridge protocol \(page 21\)](#)
- For use with BD Rhapsody™ HT Xpress 8-lane cartridge workflow, skip to the section [HT Xpress protocol \(page 22\)](#).
- See [Protocols to Follow for Cell Capture and cDNA Synthesis Step \(page 21\)](#) for information on the necessary protocol for cell capture and cDNA synthesis.

Table 8: Protocols to Follow for Cell Capture and cDNA Synthesis Step

Cartridge Type	Protocol	Step
BD Rhapsody™ Single-Lane Cartridge	With BD Rhapsody™ Scanner: <i>BD Rhapsody™ System Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Single-Cell Analysis System Protocol</i>, doc ID: 23-22951 OR Without BD Rhapsody™ Scanner: <i>BD Rhapsody™ System Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Express Single-Cell Analysis System Protocol</i>, doc ID: 23-22952	Priming and loading BD Rhapsody™ Single-Lane Cartridge
BD Rhapsody™ 8-Lane Cartridge	With BD Rhapsody™ Scanner: <i>BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol</i>, doc ID: 23-24252 OR Without BD Rhapsody™ Scanner: <i>BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol</i>, doc ID: 23-24253	Priming and loading BD Rhapsody™ 8-Lane Cartridge

Single-lane cartridge 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 \(page 21\)](#) to find the correct protocol. However, be sure to include the following modifications outlined in [steps 33–36](#) of this section.



Once loaded with cells, the cartridge should be kept on ice for the duration of the cell and bead loading incubation steps.

35. Replace sample buffer with the cold Modified Sample Buffer by adding RNase inhibitor (25 µL/mL). This is critical for RNA yield. See [Modified Sample Buffer with RNase Inhibitor for Single-Lane Cartridge \(page 21\)](#). Use the Modified Sample Buffer for Single Cell Capture protocol which requires sample buffer, including preparing cell suspension, preparing cell capture beads, and washing cell capture beads.

Table 9: Modified Sample Buffer with RNase Inhibitor for Single-Lane Cartridge

Cartridge Type	Vol. of Cold Sample Buffer (µL)	Vol. of RNase Inhibitor (µL)	Step
BD Rhapsody™ Single-Lane Cartridge	4,000	100	Preparing cell-suspension, Preparing cell capture beads, Washing cell capture beads

36. For the cell and bead loading steps, place entire cartridge on ice for the duration of the cell capture incubation (~15 minutes). Likewise, keep cartridge on ice during the bead capture incubation (~3 minutes). Try to keep cartridges flat and wipe the top and bottom of the cartridge with a lint-free wipe before

performing the scanner steps.

37. Use Lysis Buffer with 1 M DTT (5 mM final DTT concentration) and Proteinase K for the cell lysis step.
 - a. Right before lysis step, add Proteinase K (40 units/mL, add 50 µL/mL) to Lysis Buffer with 1 M DTT. See [Modified Lysis Buffer with Proteinase K for Single-Lane Cartridge \(page 22\)](#). Keep on ice.
 - b. Lyse the cells 5 minutes (instead of 2 minutes) at room temperature (15–25 °C).
 - c. Use Lysis Buffer with 1 M DTT (no Proteinase K) for the retrieval step.

Table 10: Modified Lysis Buffer with Proteinase K for Single-Lane Cartridge

Cartridge Type	Vol. of Lysis Buffer with DTT (µL)	Vol. of Proteinase K (µL)	Step
BD Rhapsody™ Single-Lane Cartridge	1,000	50	Lysis

38. Use *BD Rhapsody™ System Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Single-Cell Analysis System Protocol*, (doc ID: 23-22951) 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 BD OMICS-One™ WTA Next Amplification Kit (Catalog no. 572620).



This *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos for intracellular CITE-seq Protocol* is not compatible with BD Rhapsody™ System Targeted Assay or BD Rhapsody™ System TCR BCR Assay Kits.

For sequencing guidance, refer to one of the following protocols:

- *BD Rhapsody™ System BD OMICS-One™ WTA Next and BD® AbSeq Library Preparation Protocol*, (doc ID: 23-24492).
- *BD Rhapsody™ System BD OMICS-One™ WTA Next, BD® AbSeq, and Sample Tag Library Preparation Protocol*, (doc ID: 23-24494).

Use the latest whole transcriptome sequencing pipeline for analysis. Refer to the *BD Rhapsody™ Sequence Analysis Pipeline User's Guide*, (doc ID: 23-24580) or your local Field Applications Specialist (FAS) for more information.

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 \(page 21\)](#)s to find the correct protocol. However, be sure to include the following modifications outlined in [steps 37–40](#) of this section.



Once loaded with cells, the cartridge should be kept on ice during the duration of the cell loading and bead loading incubation steps.

39. Replace sample buffer with cold Modified Sample Buffer by adding RNase inhibitor (25 µL/mL). This is critical for RNA yield. See [Modified Sample Buffer with RNase Inhibitor for HT Xpress Cartridge \(page 23\)](#).
 - a. Use 96-deep well plate to dispense the Modified Sample Buffer for the wash steps.
 - b. Keep 96-deep-well plate with modified Sample Buffer and cells on ice while working.

- c. Use cold Modified Sample Buffer for all HT Xpress protocol steps which require sample buffer including: preparing cell suspension, preparing cell capture beads, and washing cell capture beads (see [Modified Sample Buffer with RNase Inhibitor for HT Xpress Cartridge \(page 23\)](#)).
40. For the cell loading step, keep the cartridge on ice for 8 minutes before transferring to scanner. Begin cell load scan immediately upon transfer. Likewise, keep cartridge on ice during the bead capture incubation (~3 minutes). Try to keep cartridges flat and wipe the top and bottom of the cartridge with a lint-free wipe before performing the scanner steps.

Table 11: Modified Sample Buffer with RNase Inhibitor for HT Xpress Cartridge

Cartridge Type	Vol. of Cold Sample Buffer (μL)	Vol. of RNase Inhibitor(μL)	Step
BD Rhapsody™ 8-Lane Cartridge (1 lane)	2,400	60	Preparing cell-suspension, Preparing cell capture beads, Washing cell capture beads
BD Rhapsody™ 8-Lane Cartridge (8 lanes)	19,200	480	Preparing cell-suspension, Preparing cell capture beads, Washing cell capture beads

41. Use Lysis Buffer with 1 M DTT (5 mM final DTT concentration) and Proteinase K for the lysis step.
- a. Right before the lysis step, add Proteinase K (40 units/mL, add 50 μL/mL) to Lysis Buffer with 1 M DTT. See [Modified Lysis Buffer with Proteinase K for HT Xpress Cartridge \(page 23\)](#). Keep on ice.
 - b. Lyse the cells for 4 minutes at room temperature (15–25 °C).
 - c. Place the retrieval magnet down for 1 minute before retrieval.
 - d. During retrieval: Use Lysis Buffer with 1 M DTT (no Proteinase K).

Table 12: 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)	300	15	Lysis
BD Rhapsody™ 8-Lane Cartridge (8 lanes)	2,400	120	Lysis

42. 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 OMICS-One™ WTA Next Amplification Kit (Catalog no. 572620).



- This *BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos for intracellular CITE-seq Protocol* is **not compatible** with BD Rhapsody™ System Targeted Assay or BD Rhapsody™ System TCR BCR Assay Kits.
- For sequencing guidance, refer to one of the following protocols:
 - *BD Rhapsody™ BD Rhapsody™ System BD OMICS-One™ WTA Next and BD® AbSeq Library Preparation Protocol*, (doc ID: 23-24492)
 - *BD Rhapsody™ BD OMICS-One™ WTA Next, BD® AbSeq, and Sample Tag Library Preparation Protocol*, (doc ID: 23-24494).

Use the latest whole transcriptome sequencing pipeline for analysis. Refer to the *BD Rhapsody™ Sequence Analysis Pipeline User's Guide*, (doc ID: 23-24580) or your local Field Applications Specialist (FAS) for more information.

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