BD Rhapsody™

Express Single-Cell Analysis System

Instrument User Guide

23-21332(03)
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: 214063 Rev. 1.0	2018-07	Initial release.
Doc ID: 214063 Rev. 2.0 23-21332-00	2019-02	Added information about the BD® Mouse Immune Single-Cell Multiplexing Kit.
Doc ID: 214063 Rev. 3.0 23-21332-01	2020-07	— Updated BD Biosciences technical support Email address to scomix@bdscomix.bd.com and European technical support phone number. — Updated catalog number for reverse transcriptase. — Recommended thermomixer instead of heat block for Exonuclease I inactivation.
Doc ID: 214063 Rev. 4.0 23-21332(02)	2021-11	— Removed library preparation references and associated kits. — Updated Cell Capture Bead reference to include the Enhanced Cell Bead with new Cat. No. — Removed kit component information and storage conditions from the Required kits and storage conditions section. — Removed information about BD Rhapsody™ Custom Panel. — Updated Consumable section to include only relevant information for the Cartridge Workflow. — Updated Equipment section to include only relevant information for the Cartridge Workflow. — Removed Software section. — Added a reference to how to perform reverse transcription for assays that profile BCR/TCR. — Moved the washing cell capture bead information to its own section preceding. Performing reverse transcription on Cell Capture Beads section. — Removed all references to document Single-Cell Analysis Workflow with BD Rhapsody™ Systems (Doc ID: 220524). — Added instructions to dissolve the precipitation in Lysis Buffer and DTT back to solution.
23-21332(03)	2022-11	Updated for BD Rhapsody™ Enhanced Cell Capture Beads version 2.0.

Contents

1. Introduction

About this guide

This guide provides instructions on operating the BD Rhapsody™ Express instrument and supporting materials.

To use the BD Rhapsody™ Scanner for image analysis of BD Rhapsody™ Cartridge loading, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide*. The scanner is not required in the BD Rhapsody™ Express workflow.

Single-Cell Multiomics technical publications are available for download from the BD® Single-Cell Multiomics Resource Library at scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library.

Intended use

The BD Rhapsody™ Express Single-Cell Analysis system is intended for the preparation of single-cell mRNA sequencing libraries. Proprietary BD® Molecular Indexing technology is used to count individual mRNA molecules. Cells are entirely contained in the BD Rhapsody™ Cartridge, which is a single-use consumable.

The system is intended for use by professional scientific users, such as technicians and laboratory personnel, who are trained in the operation of the BD Rhapsody™ Express instrument.

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

For more information on the purpose of the BD Rhapsody™ Express instrument, see the BD Rhapsody™ Express instrument overview (page 9).

Restrictions

Any use of the BD Rhapsody™ Express Single-Cell Analysis system other than the procedures as described in this user guide might result in damage to the instrument, loss of reagents or samples, or personal injury.

BD denies any responsibility for damage caused by the following:

- Any use of a BD Rhapsody™ Express instrument that does not comply with the procedures described in any guide used with the BD Rhapsody™ Express Single-Cell Analysis system.
- Unauthorized alterations or adjustments to instrument hardware.
- Any use of an instrument that violates locally applicable laws, rules, or regulations.
- Evidence of any deviation from intended use voids the BD Rhapsody™ instrument warranty.

Disclaimer

The instrument, external components, and consumables in the BD Rhapsody™ Express Single-Cell Analysis system are provided for research purposes only. BD disclaims all express and implied warranties, including, but not limited to, merchantability and fitness for use for a particular purpose.

Safety symbols

This topic describes the safety symbols used in this guide.

For safety and limitations, see the *BD Rhapsody™ Express Instrument Safety and Limitations Guide*.

The following table lists the safety symbols used in this guide to alert you to potential hazards.

Symbol	Meaning
	Warning. Indicates the need for the user to consult the instructions for use for important cautionary information, such as warnings and precautions that cannot, for a variety of reasons, be presented on the device itself.
	Biological hazard. All surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning surfaces. Wear suitable protective clothing, eyewear, and gloves.

Safety data sheets

This topic describes how to obtain safety data sheets (SDSs).

Before handling chemicals, read and understand the SDSs. To obtain SDSs for chemicals ordered from BD, go to regdocs.bd.com, or contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com.

Instrument technical support

This topic describes how to get technical support for the BD Rhapsody™ Express instrument.

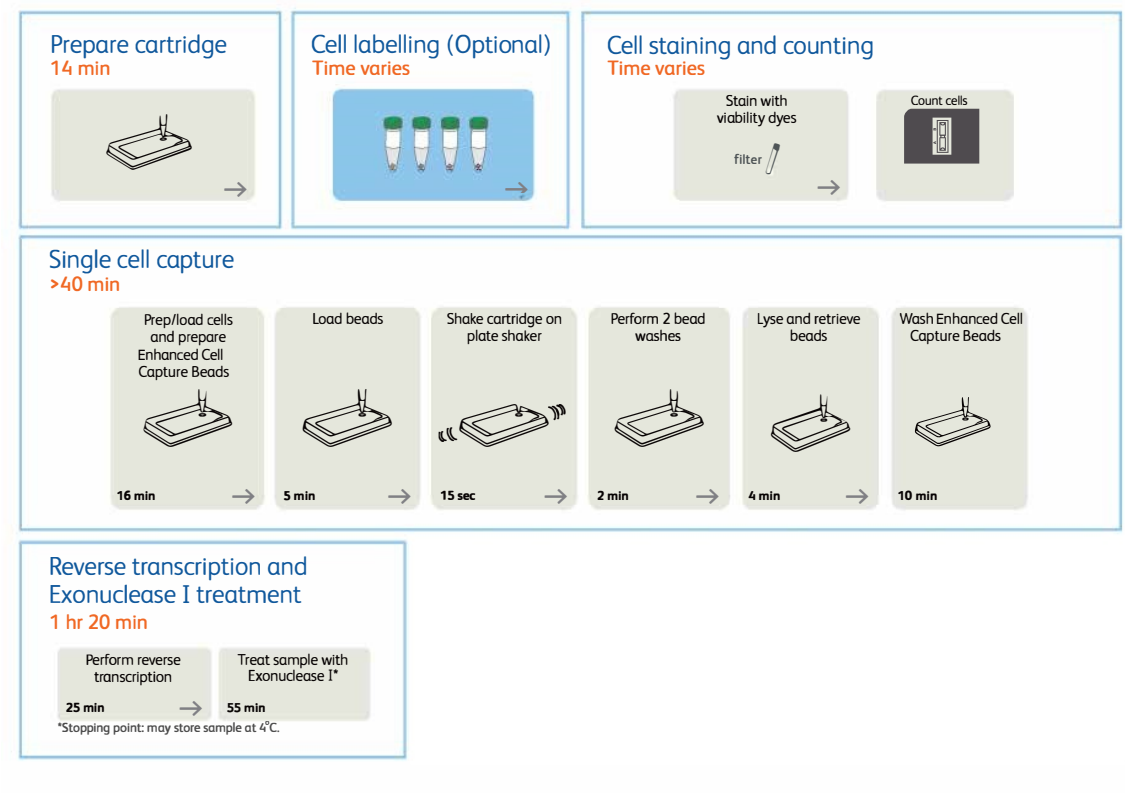
If technical assistance is required, contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com or 1.877.232.8995. You can contact technical support in Europe at help.biosciences@bd.com or +32.53.720.600.

Before contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number or lot number
- Details of recent system performance

2. Getting Started

BD Rhapsody™ Express cartridge workflow



Note: When using two cartridges, see Workflow with two BD Rhapsody™ Cartridges on page 51.

Site requirements

Dedicate two isolated work spaces in the laboratory to run high-sensitivity, single-cell sequencing experiments:

- Pre-amplification workspace
- Post-amplification workspace

For detailed site requirements and technical specifications, see the *BD Rhapsody™ Express Single-Cell Analysis System Site Preparation Guide*.

For installation of the BD Rhapsody™ Express instrument, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express instrument.*

Single-Cell Multiomics technical publications are available for download from the BD® Single-Cell Multiomics Resource Library at scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library.

Plate shaker settings

The following plate shakers have been validated for use on the BD Rhapsody™ Express Single-Cell Analysis system:

- Eppendorf ThermoMixer® C: 1,000 rpm
- Eppendorf MixMate®: 1,000 rpm
- MicroPlate Genie®: 1,600 rpm

Warning.

Use other plate shakers at your own risk. Use of other plate shakers may lead to substantial cell loss.

Thermomixer settings

Depending on the protocol, set the thermomixer from 25–95 °C and 0–1,200 rpm.

Thermal cycler setup

- Use a properly calibrated thermal cycler for 0.2-mL tubes with a maximum reaction volume of 50 µL.
- Use a heated lid set to greater than or equal to 95 °C.
- For specific instrument operation, follow the instructions provided by the manufacturer.

Pipette settings

BD Rhapsody™ P1200M and P5000M pipettes are provided pre-programmed for use during single-cell mRNA capture from the BD Rhapsody™ Cartridge. Do not change the settings, but confirm them before use.

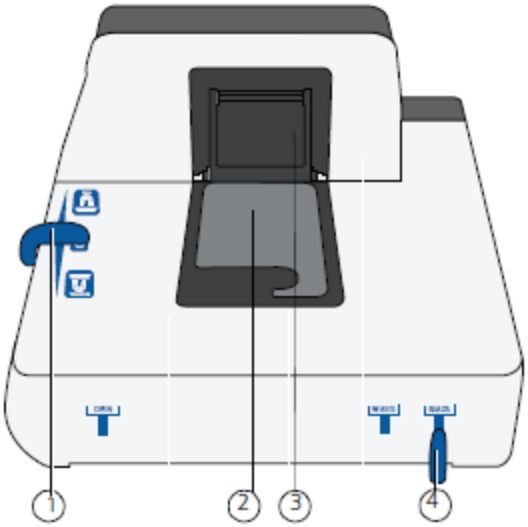
Pipette	Mode
P1200M	Prime/Treat
P1200M	Cell Load
P1200M	Bead Load
P1200M	Wash
P1200M	Lysis
P5000M	Retrieval

BD Rhapsody™ Express instrument overview

The BD Rhapsody™ Cartridge requires the use of the BD Rhapsody™ Express instrument. The station is used to load reagents, cells, and BD Rhapsody™ Enhanced Cell Capture Beads into the cartridge for bead capture and retrieval of bar-coded mRNA.

For safety and limitations of the BD Rhapsody™ Express instrument, see the *BD Rhapsody™ Express instrument Safety and Limitations Guide*.

The following figure shows the main components of the BD Rhapsody™ Express instrument for operation. For maintenance of the Express instrument, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument*.



No.	Component
1	Left slider to position: Retrieval (top) and Lysis (bottom) magnets. Slider shown in 0 (neutral) position: no magnets applied to BD Rhapsody™ Cartridge.
2	Cartridge tray to install the BD Rhapsody™ Cartridge.
3	Retrieval (top) magnet in up position.
4	Front slider to position: Waste Collection Container (WASTE), 5-mL LoBind tube for bead retrieval (BEADS), and Waste Collection Container and 5-mL LoBind tube access (OPEN).

Best practices

Good laboratory practices

- Calibrate and service pipettes every 12 months to ensure accurate sample volume transfer at each step. To clean and calibrate the pipettes, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument*.
- Unless otherwise specified, thaw reagents on ice. Store reagents at their specified storage conditions.
- Collect small volumes by briefly centrifuging samples. Brief or pulse centrifugation is <1 second.
- Gently vortex solutions containing enzymes. Minimize vortex duration, and keep the vortex speed low. Do not vortex solutions containing BD Rhapsody™ Enhanced Cell Capture Beads.
- Gently pipet cells to avoid cell stress or death.
- Work in designated pre- or post-amplification workspaces according to the protocol.
- Prepare reagent mixes in pre-amplification workspaces, and conduct amplification in post-amplification workspaces.
- Wear suitable protective clothing, eyewear, and gloves.

RNase-free technique

Prevent the introduction of exogenous RNases into samples during processing:

- Use low-retention, RNase-free pipette tips and low-binding reaction tubes when required for certain steps to prevent absorption to plastic surfaces and to minimize bead loss.
- Wear disposable gloves, and change them frequently.
- Never reuse tips or tubes.
- Keep tip boxes, reagent containers, and sample tubes closed when not in use.
- Always maintain a clean laboratory bench, and if necessary, wipe work surface with a solution of 10% (v/v) bleach.

Cell handling and cell counting

See Best practices for cell handling and cell counting on page 21.

Sterility

- Clean cell culture surfaces in the laminar flow hood with 70% (v/v) ethyl alcohol, and appropriately sterilize the surfaces.
- Use sterile serological pipettes to aseptically transfer media and cells.
- Place flasks in a cell culture hood one at a time to prevent cross-cell contamination.

BD Rhapsody™ Enhanced Cell Capture Beads

- Always keep BD Rhapsody™ Enhanced Cell Capture Beads on ice when not in use.

  Note: Do not freeze BD Rhapsody™ Enhanced Cell Capture Beads.
- For maximum recovery, do not vortex samples containing BD Rhapsody™ Enhanced Cell Capture Beads.
- Gently mix suspensions with BD Rhapsody™ Enhanced Cell Capture Beads by pipette only.

- Use low-retention tips and LoBind tubes when handling BD Rhapsody™ Enhanced Cell Capture Beads.

BD Rhapsody™ Express instrument

- The BD Rhapsody™ Express instrument contains strong magnets. Keep metal objects away from the station.
- Wipe the BD Rhapsody™ Express instrument with 70% ethyl alcohol wipes after each use.

BD Rhapsody™ cartridge

- Avoid pipetting bubbles into the cartridge. Before adding fluid to the cartridge, ensure that the pipette tip does not contain air.
- To ensure an air-tight seal with the BD Rhapsody™ P1200M and P5000M pipettes, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft.
- Cells need to be prepared as close to cell loading in the cartridge as possible. Begin cell preparation during or after the prime or substrate treatment steps, and leave the cartridge in Cartridge Wash Buffer 2 until ready to proceed with cell loading.

3. Product information

Required kits

The BD Rhapsody™ Cartridge workflow requires the following:

- BD Rhapsody™ Enhanced Cartridge Reagent Kit
- BD Rhapsody™ Cartridge Kit
- BD Rhapsody™ cDNA Kit

The cartridge-related kits listed are included in each of the following 4-pack products that also contain an amplification kit. For details of components of these 4-pack products, refer to the product insert referenced.

- Targeted mRNA and AbSeq Amplification Kit - 4 Pack
- Whole Transcriptome Analysis (WTA) Amplification Kit - 4 Pack
- TCR/BCR Amplification Kit - 4 Pack

Required reagents

Required reagents

Material	Supplier	Catalog no.
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
BD Rhapsody™ Cartridge Kit	BD Biosciences	633733
BD Rhapsody™ cDNA Kit	BD Biosciences	633773
Absolute ethyl alcohol, molecule biology grade	Major supplier	–
Nuclease-free water	Major supplier	–
Calcein AM[a]	Thermo Fisher Scientific	C1430
Propidium Iodide, 1mg/mL in Water[a]	Thermo Fisher Scientific	P3566
Trypan Blue Stain, 0.4%	Thermo Fisher Scientific	15250061
Dimethyl sulfoxide (DMSO)	Major supplier	–

a. Required for counting by viability stain and fluorescence microscope. See Counting cells by fluorescence stains and fluorescence microscopy on page 22. Protect Calcein AM and Propidium Iodide from light. Avoid multiple freeze-thaw cycles of Calcein AM. See manufacturer's storage recommendations.

Required consumables

Required consumables

Material	Supplier	Catalog no.
Gilson™ PIPETMAN™ Tipack™ Filter Tips, 100-1200 µL for BD Rhapsody™ P1200M pipette	Thermo Fisher Scientific	F171803G
Gilson™ PIPETMAN™ Tipack™ Filter Tips, 500-5000 µL for BD Rhapsody™ P5000M pipette	Thermo Fisher Scientific	F161370G
Falcon® Tube with Cell Strainer Cap	Corning	352235
Falcon tubes, 5-mL Round Bottom Polystyrene Test Tube[a]	Corning	352054
DNA LoBind® Tubes, 1.5-mL	Eppendorf	30108051
DNA LoBind® Tubes, 5.0-mL[b]	Eppendorf	30108310
Low-retention, filtered pipette tips (10 µL, 200 µL, 1000 µL)	Major supplier	–
10 mL sterile serological pipettes	Major supplier	–
Premoistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol	Major supplier	–
Lint-free wipes	Major supplier	–

a. Required for sample multiplexing and/or antibody-oligonucleotide labeling.
b. These are the Bead Retrieval Tubes to be used with the BD Rhapsody™ Express instrument.

Suggested consumables

Suggested consumables

Consumable item	Supplier	Catalog no.
Improved Neubauer Hemocytometer[a]	INCYTO	DHC-N01-5
Cell Counting Slides for TC20™ Cell Counter	Bio-Rad	1450011

a. For cell counting under a microscope.

Required equipment

Supply pre- and post-amplification work spaces with the required equipment. You might need two sets of some equipment.

Required equipment

Equipment	Supplier	Catalog no.
BD Rhapsody™ Express Instrument[a]	BD Biosciences	633702
BD Rhapsody™ P1200M pipette[a]	BD Biosciences	633704
BD Rhapsody™ P5000M pipette[a]	BD Biosciences	633705
Large magnetic separation stand	V&P Scientific, Inc.	VP 772FB-1
Clear acrylic cylinder adapter for 15-mL tube adapter[b]	V&P Scientific, Inc.	VP 772FB-1A
Microcentrifuge for 1.5–2.0-mL tubes[c]	Major supplier	–
Microcentrifuge for 0.2-mL tubes	Major supplier	–
Centrifuge and rotor with adapters for 5-mL Falcon tubes and 15-mL tubes	Major supplier	–
Pipet-Aid	Major supplier	–
Eppendorf ThermoMixer® C	Eppendorf	5382000023
SmartBlock™ Thermoblock 1.5-mL	Eppendorf	5360000038
Plate Shaker for cartridge workflow: SmartBlock™ plates (for ThermoMixer C) Or, Eppendorf MixMate® Or, MicroPlate Genie™	 Eppendorf Eppendorf Scientific Industries, Inc.	 5363000039 22674200 SI-0400
Water bath OR incubator at 37 °C	Major supplier	–
Pipettes (P10, P20, P200, P1000)[c]	Major supplier	–
Vortexer[c]	Major supplier	–
Digital timer[c]	Major supplier	–
6-Tube Magnetic Separation Rack for 1.5-mL tubes[c]	New England Biolabs	S1506S
Low-profile magnet separation stand for 0.2-mL, 8-strip tubes	V&P Scientific, Inc. Or, Clontech	VP 772F4-1 635011

a. Part of the BD Rhapsody™ Single-Cell Analysis system. Items can be ordered separately.
b. Holds 5-mL LoBind tube in magnet.
c. Provide equipment in both pre- and post-amplification workspaces.

Suggested equipment

Suggested equipment

Equipment	Supplier	Catalog no.
TC20™ Automated Cell Counter	Bio-Rad	1450102
Fluorescence widefield microscope capable of the following excitation/emission: • Channel: 494 nm/517 nm • Channel: 533 nm/617 nm	Major supplier	–
Brightfield microscope	Major supplier	–
Phase-contrast microscope	Major supplier	–

4. Preparing the BD Rhapsody™ Cartridge

For a streamline protocol of the cartridge workflow intended for expert users, refer to “Capturing Single Cell mRNA” in the *BD Rhapsody™ Cartridge Single-Cell Analysis System Ultra-Quick Reference*.

Priming the BD Rhapsody™ Cartridge

Before you begin

After opening the cartridge packet, ensure that you prepare the cartridge within 1 day before use. If you leave the cartridge at 2–8 °C, equilibrate to room temperature (15–25 °C) for 5 minutes.

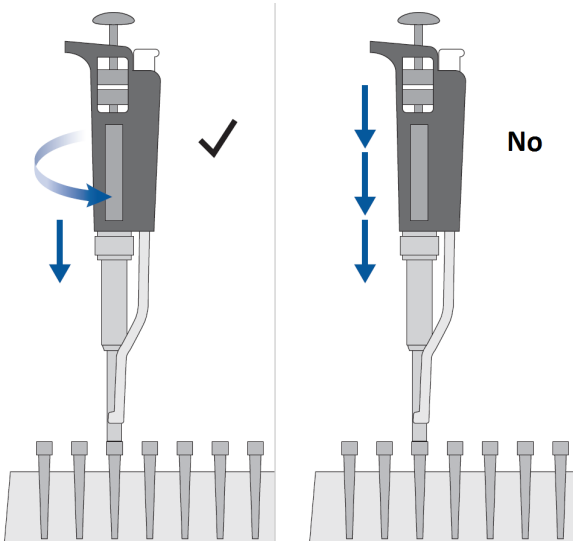
Visually inspect the Lysis Buffer for any precipitation. If precipitation is present, incubate the Lysis Buffer at room temperature (15–25 °C) for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, place the Lysis Buffer on ice.

If cell preparation takes 4 hours or longer, begin preparing cells before cartridge preparation.

- Equilibrate these reagents at room temperature (15–25 °C) within 30 minutes before use:
  - Cartridge Wash Buffer 1
  - Cartridge Wash Buffer 2
- Place these reagents on ice:
  - Sample Buffer
  - 1 M DTT
  - Lysis Buffer
- Review pipette settings and operation. See Pipette settings on page 8.
- For the use of two cartridges, see Workflow with two BD Rhapsody™ Cartridges on page 51.

Procedure

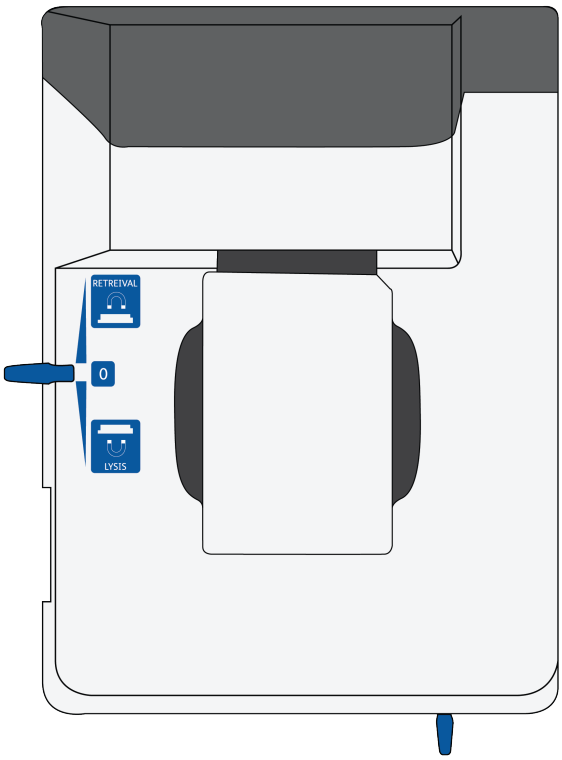
To ensure an air-tight seal with the BD Rhapsody™ P1200M or P5000M pipette, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft:



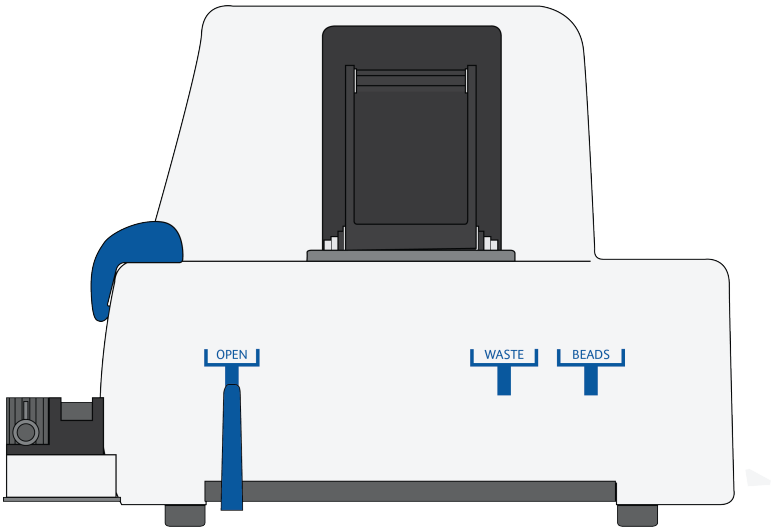
Avoid introducing bubbles while pipetting into the BD Rhapsody™ Cartridge.

Note: Change pipette tips before every pipetting step.

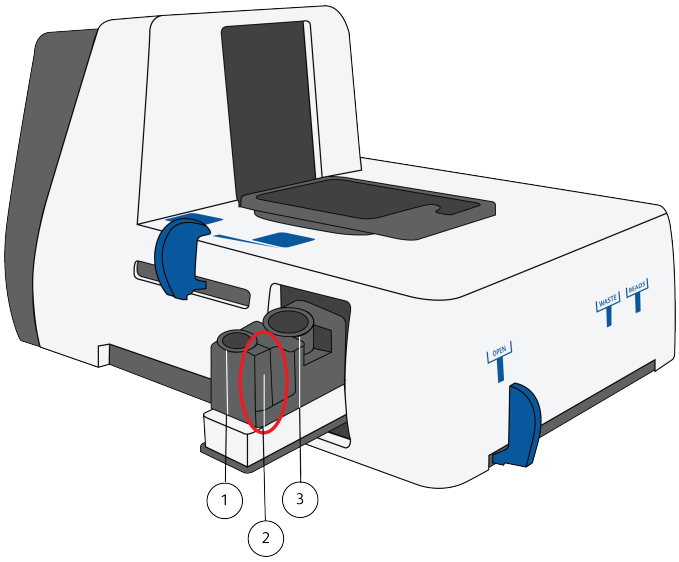
1. Move the left slider to the middle (0) position on the BD Rhapsody™ Express instrument. The Retrieval (top) magnet and Lysis (bottom) magnets are away from the cartridge tray:



2. Move the front slider to **OPEN**:

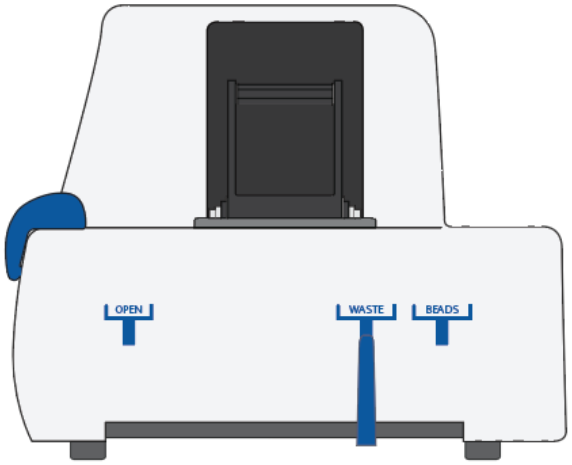


3. Remove the cap of a Waste Collection Container, and insert both the container and a new 5-mL LoBind tube for bead retrieval into the appropriate slots in the drawer. Secure the cap of the 5-mL LoBind tube to the holder:



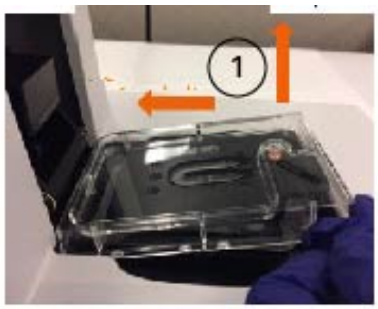
No.	Component
1	5-mL LoBind tube for bead retrieval
2	Secure cap of 5-mL LoBind tube here
3	Waste Collection Container

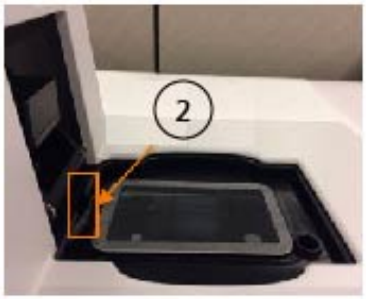
4. Move the front slider to **WASTE**:



5. Push the cartridge into the far end of the tray to match the cartridge and tray notches. Lay the cartridge flat, and release it. Ensure that the cartridge is flat in the tray and the barcode faces out.

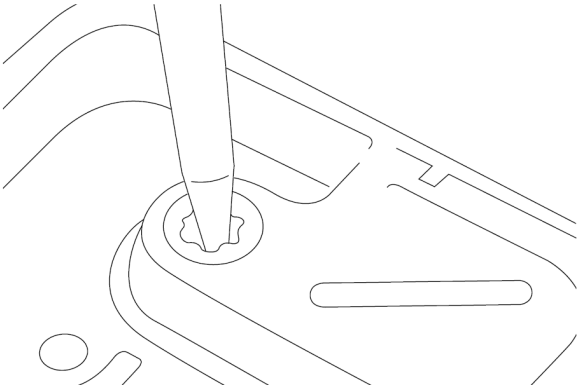
Note: To remove the cartridge from the BD Rhapsody™ Express instrument, push in the cartridge, and lift it from the tray:





No.	Component
1	Push and lift
2	Metal clip

Note: Before loading the reagent into the cartridge, align the pipette tip with the inlet hole of the gasket, and then press down on the P1200M pipette to seal the pipette tip against the gasket and avoid leaks:



Note: In **Prime/Treat** mode, press the button once to aspirate 700 µL, and press the button again to dispense 700 µL.

6. Load the cartridge with 700 µL of 100% (absolute) ethyl alcohol using the P1200M pipette in **Prime/Treat** mode.
7. Load the cartridge with 700 µL of air using the P1200M pipette in **Prime/Treat** mode.
8. Load the cartridge with 700 µL of Cartridge Wash Buffer 1 with the P1200M pipette in **Prime/Treat** mode.
9. Leave the cartridge on the tray at room temperature (15–25 °C) for 1 minute.

Treating the surface of the cartridge

1. Load the cartridge with 700 µL of air using the P1200M pipette in **Prime/Treat** mode.
2. Load the cartridge with 700 µL of Cartridge Wash Buffer 1 using the P1200M pipette in **Prime/Treat** mode.
3. Leave the cartridge on the tray at room temperature (15–25 °C) for 10 minutes.
4. Load the cartridge with 700 µL of air using the P1200M pipette in **Prime/Treat** mode.
5. Load the cartridge with 700 µL of Cartridge Wash Buffer 2 using the P1200M pipette in **Prime/Treat** mode.

   Stopping point: The cartridge can be stored at room temperature (15–25 °C) for up to 4 hours. You can leave the cartridge on the tray. The performance of the cartridge has not been validated at room temperature (15–25 °C) storage for more than 4 hours.

6. Prepare a single-cell suspension.

5. Counting cells

Best practices for cell handling and cell counting

Cell handling

- Minimize cell handling to reduce cell loss and decline in cell viability.
- Keep cells on ice when not handling them.
- Optimize centrifugation conditions according to cell number and cell phenotype to see a cell pellet.
- For high cell recovery, know the position of the cell pellet in the tube after centrifugation.

Cell counting

Note: It is critical to obtain an accurate cell count of the cell suspension in order for the sample to be analyzed with the BD Rhapsody™ Express Single-Cell Analysis system. If you overestimate the cell count, you will underload cells in the BD Rhapsody™ Cartridge. If you underestimate the cell count, you will overload the cartridge and generate excessive multiplets. Validate that the cell quantitation method that you use is accurate and reliable for your cells of interest.

- Filter cells to remove clumps and debris to ensure accurate cell counting. Debris in suspensions of small cells can lead to overestimated cell counts.
- Avoid pipetting low volumes of cells (<2 µL).
- Concentrate cells as needed, and then recount them using one of the recommended methods in Cell counting methods on page 22.
- Do not rely on FACS-based counts, because cell concentration might be overestimated by this method. Always re-count cells after FACS using one of the recommended methods in Cell counting methods on page 22.
- If you are counting cells for the first time or if the cells of interest are of varying sizes, use at least two different counting methods. Counting by fluorescence is particularly appropriate with peripheral blood mononuclear cells (PBMCs), samples with debris, or samples with a significant fraction of non-nucleated cells.
- Treat cells with viability stain(s), and then count them by using one of the recommended methods. See Cell counting methods on page 22.
- Be aware and adjust for limitations in cell counting methods, because there can be variations in cell counts. When using automated cell counters, be sure to use the appropriate settings validated for the cells of interest; for example, cell size, brightness, and circularity. When manually counting cells under the microscope, cell counts might vary due to brightness and focus.
- For accurate cells counts, take three separate aliquots from completely suspended cells, and count each aliquot once. Use the median cell concentration.
- Count cells that have been diluted to the recommended range for the cell counting method. See Assessing cell concentration on page 24. If the concentration is outside that range, dilute or concentrate the cells, accordingly, and recount them.
- Calculate the cell concentration for the total number of cells, not just for live cells. Use the total number of cells to determine the volume of stock cell suspension loaded into the cartridge.

Cell counting methods

Count cells by one of three methods:

- Counting cells by fluorescence stains and fluorescence microscopy on page 22
- Counting cells by Trypan Blue staining and brightfield microscopy on page 23
- Counting cells by automated cell counting on page 24

If you use fluorescent stains and fluorescent microscopy, thaw Calcein AM. Once at room temperature (15–25 °C), resuspend Calcein AM (1 mg) in 503.0 µL of DMSO for a final stock concentration of 2 mM. Keep the stock solution at room temperature (15–25 °C), and protect it from light immediately before and during use while cell counting. Store Calcein AM according to the manufacturer's storage recommendations.

Counting cells by fluorescence stains and fluorescence microscopy

Use a suitable stain to detect cell viability by fluorescence under the microscope [excitation/emission: 494 nm/517 nm and (533 nm/617 nm)]. We recommend the use of Calcein AM and Propidium Iodide.

Note: Protect Calcein AM and Propidium Iodide from light until ready to use.

1. If the cells are not already resuspended in 620 µL of cold Sample Buffer, centrifuge the cell suspension at 400 × g for 5 minutes, aspirate the supernatant, and leave ~20 µL of the residual supernatant. Add 620 µL total volume of cold Sample Buffer, and then proceed with cell staining in **step 2**.
2. Add 3.1 µL of 2 mM Calcein AM and 4.1 µL of 1 mg/mL Propidium Iodide in Water to the 620 µL volume of cell suspension.
3. Gently pipet the suspension up and down to mix well.
4. Incubate the suspension in the dark in a water bath, incubator, or heat block at 37 °C for 5 minutes.
5. Pass the cells through a Falcon® Tube with Cell Strainer Cap.

   Note: For low abundance or low volume samples, filtering is optional at this step. We recommend filtering the final sample or pooled sample (for multiplexed samples) before loading cells into the cartridge.

   Note: Count cells immediately.

   Note: Keep cells on ice, and protect them from light.
6. Gently mix cells well by pipette, and then pipet 10 µL from the center of the cell suspension into one chamber of the INCYTO™ disposable hemocytometer or non-disposable hemocytometer with coverslip.
7. Count the green/Calcein AM-positive (live) and red/Propidium Iodide-positive (dead) cells in the four corner 1 × 1 mm^2 squares of the hemocytometer. (To view the grid of the Neubauer Improved hemocytometer, see incyto.com.) If the live + dead cell count is:
   - 100–640 cells total in four 1 × 1 mm^2 squares, proceed to **step 8**.
   - <100 total in four 1 × 1 mm^2 squares: Count all nine 1 × 1 mm^2 squares. If the cell count is <100 after counting nine squares, count cells in another hemocytometer or centrifuge the cell sample, and recount the concentrated sample.
   - >160 cells/1 × 1 mm^2 square, dilute the cell suspension in cold Sample Buffer, and recount the cells.
8. Calculate the concentration of the stock cell suspension and the cell viability:

   Viable cells/µL = No. of live cells ÷ no. squares counted x 10

   Total no. of cells/µL = (No. live cells + no. dead cells) ÷ no. squares counted x 10

Percent viable cells = (Viable cells/µL) ÷ (total cells/µL) x 100

9. Record the total cell concentration (cells/µL), live cell concentration (cells/µL), and percent viability.
10. Repeat **steps 1–9** for each new sample if multiplexing samples. Assess total cell concentration, live cell concentration, and percent viability from three independent aliquots of your sample. Identify the aliquot with the median total cell concentration and use values (total cell concentration, live cell concentration, and viability) from this replicate.
11. If using a disposable hemocytometer, dispose of it according to local safety regulations. If using a non-disposable hemocytometer, clean it according to local safety regulations.
12. Proceed immediately to Assessing cell concentration on page 24.

Counting cells by Trypan Blue staining and brightfield microscopy

1. Pass the cells through a Falcon Tube with Cell Strainer Cap.

   Note: For low-abundance or low-volume samples, filtering is optional at this step. We recommend filtering the final sample or pooled sample (for multiplexed samples) before loading cells into the cartridge.

2. Gently mix cells well by pipette, and then pipet 10 µL from the center of the suspension into a new 1.5-mL LoBind tube.
3. Add 10 µL of 0.4% Trypan Blue Stain.
4. Gently mix the cells by pipette, and then pipet 10 µL from the center of the stained cell suspension into one chamber of the INCYTO disposable hemocytometer or a non-disposable hemocytometer with coverslip.
5. If counting multiple aliquots of sample, use a new pipette tip each time to repeat steps 2–3 two more times for a total of three tubes of cells with Trypan Blue Stain.
6. Count the unstained (live) and stained (dead) cells in the four corner 1 × 1 mm^2 squares of the hemocytometer (To view the grid of the Neubauer Improved hemocytometer, see incyto.com.) If the live + dead cell count is:
   - 100–640 cells total in four 1 × 1 mm^2 squares, proceed to **step 7**.
   - <100 total in four 1 × 1 mm^2 squares: Count all nine 1 × 1 mm^2 squares. If the cell count is <100 after counting nine squares, count cells in another hemocytometer or centrifuge the cell sample, and recount the concentrated sample.
   - >160 cells/1 × 1 mm^2 square, dilute the cell suspension in cold Sample Buffer, and recount the cells.
7. Calculate the concentration of the stock cell suspension and the cell viability:

   Viable cells/µL = No. live cells ÷ no. squares counted x 2 x 10

   Total cells/µL = (No. live cells + no. dead cells) ÷ no. squares counted x 2 x 10

   Percent viable cells = (Viable cells/µL) ÷ (Total cells/µL) x 100

8. Record the total cell concentration (cells/µL), live cell concentration (cells/µL), and percent viability.
9. Repeat **steps 1–8** for each sample if multiplexing samples. Assess total cell concentration, live cell concentration, and percent viability from three independent aliquots of your sample. Identify the aliquot with the median total cell concentration, and use values (total cell concentration, live cell concentration, and viability) from this replicate.
10. If using a disposable hemocytometer, dispose of it according to local safety regulations. If using a non-disposable hemocytometer, clean it according to local safety regulations.
11. Proceed immediately to Assessing cell concentration on page 24.

Counting cells by automated cell counting

Follow the manufacturer's instructions for your specific automated cell counter. Assess total cell concentration, live cell concentration, and percent viability from three independent aliquots of your sample. Identify the aliquot with the median total cell concentration and use values (total cell concentration, live cell concentration, and viability) from this replicate.

Assessing cell concentration

1. Find the appropriate range of cell concentrations for precision cell counting:

Cell concentration ranges of sample for precision counting

Count method	Countess™ II Automated Cell Counter (cells/µL)		TC20™ Automated Cell Counter (cells/µL)	Manual counts (cells/µL)[a]	
Stain	**Trypan Blue Stain**	**Fluorescence**	**Trypan Blue Stain**	**Trypan Blue Stain**	**Fluorescence**
Minimum[b]	500	250	500	222	111
Maximum	10,000[c]	10,000[c]	10,000[c]	3,200[d]	1,600[d]

a. Cell counting in four 1 × 1 mm^2 squares of the hemocytometer. If the live + dead cell count is <100, count all nine 1 × 1 mm^2 squares. If the cell count is <100 after counting nine squares, count cells in another hemocytometer, or centrifuge the cell sample and recount the concentrated sample. If the number of cells is outside that range, dilute or concentrate the cells as needed.

b. Minimum cell concentrations are based on a calculated precision of <±10%.

c. Maximum cell concentrations for automated cell counts are based on the T20 and Countess II user guides.

d. Maximum cell concentrations for manual counts is assuming that a user can count up to N = 10 cells per 250 × 250 μm^2 square and up to N = 160 per 1 × 1 mm^2 square. If N ≥160 cells per 1 × 1 mm^2 square, dilute the sample, and recount it.

2. Proceed as follows:
   - If the cell concentration of every sample is within the range of the counting method, proceed immediately to single-cell capture. If the cell concentration is below range, centrifuge the cell sample, and recount the concentrated sample.
   - If the cell concentration of any sample is higher than the maximum concentration of the counting method, dilute the cell suspension in cold Sample Buffer to within the recommended cell concentration range, and recount the cells. See Cell counting methods on page 22.

Preparing a single-cell suspension for cartridge loading

1. Determine the desired number of cells to capture in the BD Rhapsody™ Cartridge. The following table lists the estimated multiplet rate based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads.

Estimated multiplet rate based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads

Number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads (target)[a]	Estimated multiplet rate (%)
100	0.0
500	0.1
1,000	0.2
2,000	0.5
3,000	0.7
4,000	1.0
5,000	1.2
6,000	1.4
7,000	1.7
8,000	1.9
9,000	2.1
10,000	2.4
11,000	2.6
12,000	2.8
13,000	3.1
14,000	3.3
15,000	3.5
16,000	3.8
17,000	4.0
18,000	4.2
19,000	4.5
20,000	4.7
a. The number of cells sequenced might be less than the number of cells captured due to bead loss during handling, panel choice, and sample composition. The validated range of cells sequenced is 100–10,000 cells.	

2. Determine the pooling ratio of samples to load onto the BD Rhapsody™ Cartridge. For example, if two samples were labeled using the BD Rhapsody™ Single-Cell Multiplexing Kit, and the samples will be pooled in equal proportion, the pooling ratio for each sample is 0.5. If only one sample is used, the pooling ratio is 1.
3. Calculate the volume, *V*, for each sample needed to prepare the pooled single-cell suspension:

$V = N \times P \times 1.36 / C$

where:

V = volume of sample needed (µL)

N = desired number of captured cells in cartridge

P = pooling ratio

C = total cell concentration (cells/µL)

Example

On a BD Rhapsody™ Cartridge, you want to capture 10,000 cells that are pooled equally of Sample A and Sample B.

N = desired number of captured cells in cartridge = 10,000

P_A = sample A pooling ratio = 0.5

P_B = sample B pooling ratio = 0.5

C_A = sample A total cell concentration = 200 cells/µL

C_B = sample B total cell concentration = 400 cells/µL

Volume of sample A needed = 10,000 cells x 0.5 x 1.36 / 200cells/µL= 34 µL

Volume of sample B needed = 10,000 cells x 0.5 x 1.36 / 400cells/µL= 17 µL

4. Calculate the sum of all of the sample volumes, Vn, to be used in the cell suspension. Using the example in **step 3**:

   Vn = 34 µL + 17 µL= 51 µL

5. Calculate the volume of cold Sample Buffer, B, that is needed to bring the final volume of cell suspension to 650 µL. Using the example in step 4:

   B = 650 µL – 51 µL= 599 µL

   Note: For low-abundance samples, the final cell suspension can be prepared in 610 µL of cold Sample Buffer.

6. According to the calculations in **steps 2–5**, prepare the cell suspension in cold Sample Buffer in a new 1.5-mL LoBind tube.

   Note: Ensure stock solution is well suspended by gentle pipet-mixing before pooling.

7. If the samples were not filtered before counting cells, filter through a Falcon® tube with Cell Strainer Cap.
8. Proceed immediately to Processing cells with the BD Rhapsody™ Express Single-Cell Analysis system on page 27.

6. Processing cells with the BD Rhapsody™ Express Single-Cell Analysis system

Loading cells in the BD Rhapsody™ Cartridge

Best practices

- Always use low-retention filtered pipette tips and LoBind tubes.
- Perform single-cell capture and cDNA synthesis in a preamplification workspace.
- Prepare cells as close to cell loading as possible. Keep other reagents, including Sample Buffer on ice unless instructed otherwise.
- Change pipetting tips before every pipetting step.

Before you begin

- Prime and treat the BD Rhapsody™ Cartridge. See Preparing the BD Rhapsody™ Cartridge on page 16.
- Thaw reagents (not enzymes) in the BD Rhapsody™ cDNA Kit at room temperature (15–25 °C), and then place them on ice. Keep enzymes at –25 °C to –15 °C.
- Prepare a single-cell suspension for cartridge loading.
- Place these reagents on ice:
  - Sample Buffer
  - 1 M DTT
  - Lysis Buffer
  - BD Rhapsody™ Enhanced Cell Capture Beads

Loading cells into the cartridge

Note: To ensure an air-tight seal with the BD Rhapsody™ P1200M and P5000M pipettes, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft.

1. Load the cartridge on the tray with 700 µL of air using the BD Rhapsody™ P1200M pipette in Prime/Treat mode.
2. Change the mode of the BD Rhapsody™ P1200M pipette to Cell Load.
3. With a manual pipette, gently pipet the cell suspension up and down to mix. Immediately proceed to cell loading.
4. On the BD Rhapsody™ P1200M pipette, press the pipette button once to aspirate 40 µL of air, immerse the pipette tip in cell suspension, and then press the button again to aspirate 575 µL of cold cell suspension.
5. Insert the tip of the pipette perpendicular to the port, seal the pipette tip against the gasket, and then press the button a third time to dispense 615 µL of air and cells.

   Note: Air bubbles that might appear at the inlet or outlet of the cartridge do not affect cartridge performance.

6. Leave the cartridge with loaded cells on the tray at room temperature (15–25 °C) for 15 minutes. During incubation on the laboratory bench, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See Preparing BD Rhapsody™ Enhanced Cell Capture Beads on page 28.

Preparing BD Rhapsody™ Enhanced Cell Capture Beads

Before you begin

- Prepare the pre-amplification workspace for preparation of the BD Rhapsody™ Enhanced Cell Capture Beads for the BD Rhapsody™ Cartridge.
- Keep the BD Rhapsody™ Enhanced Cell Capture Beads on ice before use.
- For maximum recovery, do not vortex samples containing BD Rhapsody™ Enhanced Cell Capture Beads.
- Gently mix suspensions with BD Rhapsody™ Enhanced Cell Capture Beads by pipette only.

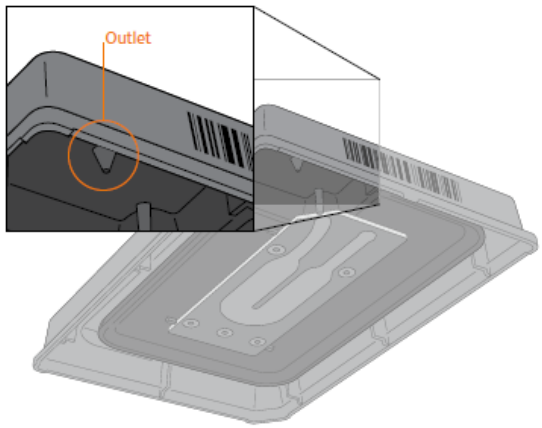
Preparing BD Rhapsody™ Enhanced Cell Capture Beads

Use low-retention pipette tips and LoBind tubes when handling BD Rhapsody™ Enhanced Cell Capture Beads.

1. Place the tube with BD Rhapsody™ Enhanced Cell Capture Beads on the 1.5-mL tube magnet for 1 minute.
2. Carefully remove and appropriately discard the storage buffer without disturbing the beads and while leaving the tube on the magnet.
3. Remove the tube from the magnet, and then pipet 750 µL of cold Sample Buffer into the tube of beads.
4. Pipet the bead suspension up and down to mix.
5. Keep the beads on ice.

Loading BD Rhapsody™ Enhanced Cell Capture Beads on to the BD Rhapsody™ Cartridge

1. Change the mode of the BD Rhapsody™ P1200M pipette to **Prime/Treat**.
2. Load the cartridge with 700 µL of air using the BD Rhapsody™ P1200M pipette in Prime/Treat mode.
3. Change the mode of the BD Rhapsody™ P1200M pipette to Bead Load.
4. Use a P1000 standard pipette to gently pipet the BD Rhapsody™ Enhanced Cell Capture Beads in cold Sample Buffer up and down to mix, and, using the BD Rhapsody™ P1200M pipette in Bead Load mode, immediately load the cartridge with 630 µL of beads.
5. Let the beads settle in the cartridge on the tray at room temperature (15–25 °C) for 3 minutes.
6. Place the cartridge on the plate shaker, and secure it on the plate adapter.
7. Shake the cartridge at room temperature (15 °C to 25 °C) for 15 seconds according to the settings for the plate shaker:
   - Eppendorf ThermoMixer® C: 1,000 rpm
   - Eppendorf MixMate®: 1,000 rpm
   - MicroPlate Genie®: 1,600 rpm. Set an external timer for 15 seconds.
8. Remove the cartridge from the plate shaker and, keeping the cartridge level, blot away the outlet drip from the bottom of the cartridge with a lint-free wiper.



9. Return the cartridge to the tray of the BD Rhapsody™ Express instrument, and wait 30 seconds.
10. Change the mode of the BD Rhapsody™ P1200M pipette to **Wash**.

    Note: In **Wash** mode, press the button once to aspirate 720 µL of air or reagent. After aspiration, insert the tip into the cartridge, and then press the button once to dispense 700 µL of air or liquid. After removing the pipette tip from the cartridge inlet, press the button once to dispense the remaining 20 µL of air or liquid before ejecting the pipette tip.
11. Load the cartridge with 700 µL of air using the BD Rhapsody™ P1200M pipette in **Wash** mode.
12. Load the cartridge with 700 µL of cold Sample Buffer using the BD Rhapsody™ P1200M pipette in **Wash** mode.
13. Repeat **steps 11–12** once for a total of two washes.

Lysing cells and retrieving BD Rhapsody™ Enhanced Cell Capture Beads

Lysing the cells

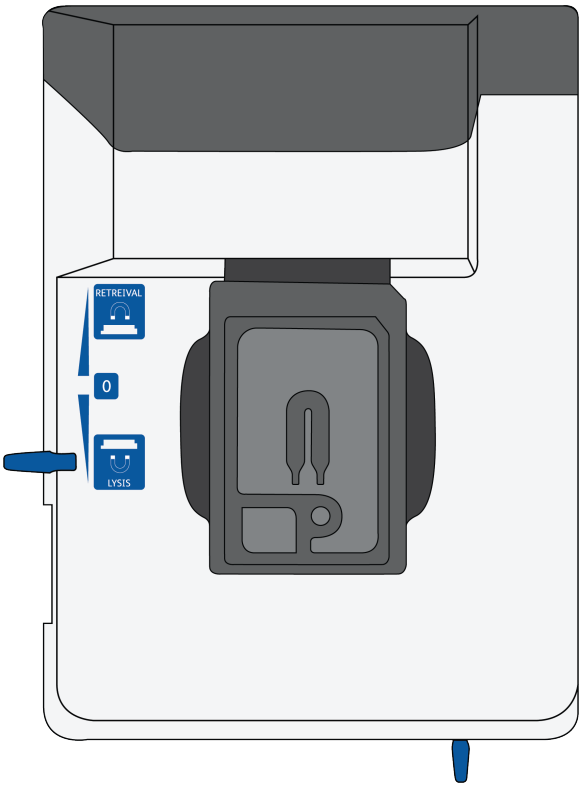
Avoid bubbles.

Open the DTT tube while holding it vertically. The solution is overlain with an inert/non-oxygen-containing gas. A non-vertical tube will allow the inert gas to pour off. If you are not loading the 4 cell cartridges at the same time, after opening the DTT tube once, seal and store it at -20 °C.

1. Add 75.0 µL of 1 M DTT to one bottle of 15 mL Lysis Buffer, and then check the Add DTT box on the Lysis Buffer label.

   Note: Use the Lysis Buffer with DTT within 24 hours, and then discard.
2. Briefly vortex the lysis mix, and place it on ice.

3. Move the left slider to **LYSIS**. The (bottom) magnet is in the up position and is in contact with the cartridge.

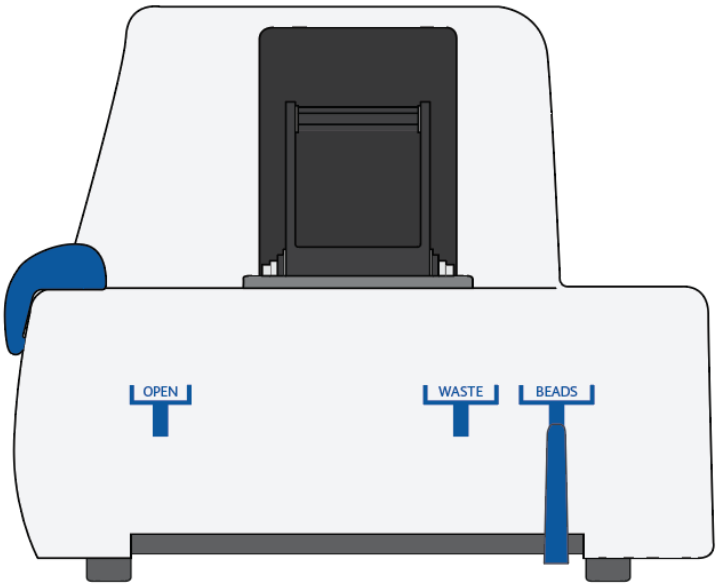


4. Change the mode on the BD Rhapsody™ P1200M pipette to **Lysis**.
5. Load the cartridge with 550 µL of Lysis Buffer with DTT using the BD Rhapsody™ P1200M pipette in **Lysis** mode.
6. Leave the cartridge at room temperature (15–25 °C) on the tray for 2 minutes.

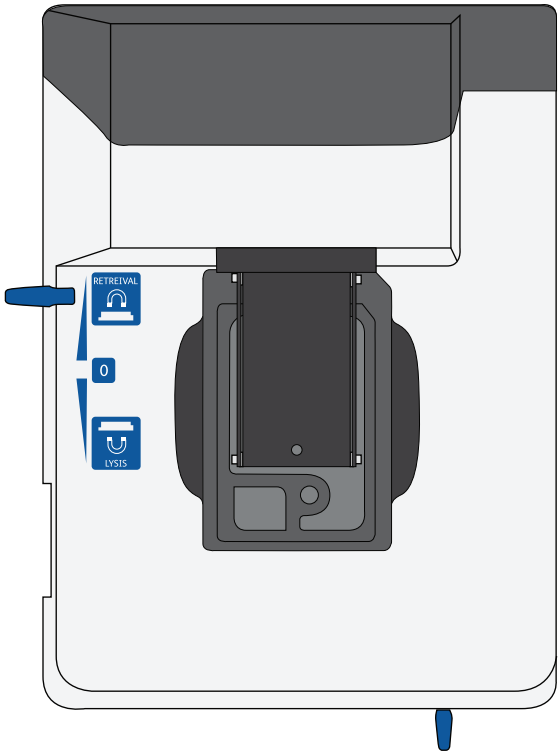
Note: Maintain recommended lysis time for best performance.

Retrieving the BD Rhapsody™ Enhanced Cell Capture Beads from the cartridge

1. Ensure that a 5-mL LoBind tube was inserted into the drawer for bead retrieval.
2. Confirm that the mode on the BD Rhapsody™ P5000M pipette is **Retrieval**. The pipette is locked into this single mode.
3. Move the front slider to **BEADS**.



4. Move the left slider to **RETRIEVAL**. The (top) magnet is in the down position and is in contact with the cartridge.



5. Leave the Retrieval magnet in the down position for 30 seconds.
6. Use the BD Rhapsody™ P5000M pipette to aspirate 5,000 µL of Lysis Buffer with DTT.
7. Press down firmly on the BD Rhapsody™ P5000M pipette to seal the pipette tip against the gasket of the cartridge to avoid leaks.
8. Move the left slider to the middle (0) position, and immediately load the cartridge with 4,950 µL of Lysis Buffer with DTT using the BD Rhapsody™ P5000M pipette. The Retrieval (top) magnet is in its full up position and is away from the cartridge.

   The BD Rhapsody™ Enhanced Cell Capture Beads are collected in the 5-mL LoBind tube.
9. Remove the pipette tip from the inlet gasket of the cartridge before pressing the dial button once to purge the tip. Discard the pipette tip.
10. Move the front slider to **OPEN**, and then remove and cap the 5-mL LoBind tube.
11. Uncap the 5-mL LoBind tube, and place it on the large magnetic separation stand fitted with the 15-mL tube adapter for 1 minute.
12. Proceed immediately to Performing reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads on page 33 to process the beads and begin reverse transcription.
13. Appropriately dispose of the BD Rhapsody™ Cartridge according to biosafety level (BSL).

	Biological hazard.

   - BSL-1. Discard the cartridge in a recycle container.
   - BSL-2. Discard the cartridge in a biosafety waste container.

     Dispose of waste using proper precautions and in accordance with local regulations. For more information, see Waste on page 52.
14. Appropriately dispose of the waste in the Waste Collection Container.
15. Appropriately dispose of the Lysis Buffer with DTT.
16. Wipe the Express instrument with 10% (v/v) bleach or 70% (v/v) ethyl alcohol. See the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument*.

Washing the BD Rhapsody™ Enhanced Cell Capture Beads

Note: Keep the BD Rhapsody™ Enhanced Cell Capture Beads cold during washes.

Note: Use low-retention tips to handle BD Rhapsody™ Enhanced Cell Capture Beads.

1. After the 1 minute incubation on the large magnet [see Lysing cells and retrieving BD Rhapsody™ Enhanced Cell Capture Beads on page 29] and while leaving the 5-mL LoBind® tube on the large magnet, use a pipette to carefully remove all but ~1 mL of supernatant without disturbing the beads.
2. Remove the tube from the large magnet, resuspend the ~1 mL beads by gently pipetting the suspension up and down, and then transfer the bead suspension to a new 1.5-mL LoBind® tube.
3. If the beads remain in the 5-mL LoBind tube, pipet an additional 0.5 mL of Lysis Buffer with DTT into the 5 mL tube, rinse the 5 mL tube, and transfer the suspension to the 1.5-mL LoBind® tube of beads.

4. Place the tube on the 1.5-mL tube magnet for up to and including 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.

   Note: Avoid leaving Lysis Buffer or bubbles in the tube. Lysis Buffer might cause the reverse transcription reaction to fail.

5. Remove the 1.5-mL LoBind® tube from the magnet, and then pipet 1.0 mL of cold Bead Wash Buffer into the tube. Gently mix the suspension by pipette only. Do not vortex.
6. Place the tube on the 1.5-mL tube magnet for up to and including 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
7. Remove the 1.5-mL LoBind® tube from the magnet, and then pipet 1.0 mL of cold Bead Wash Buffer into the tube. Gently mix the suspension by pipette only, and place the tube on ice. Do not vortex.

Note: Start reverse transcription within 30 minutes after washing retrieved BD Rhapsody™ Enhanced Cell Capture Beads with Bead Wash Buffer.

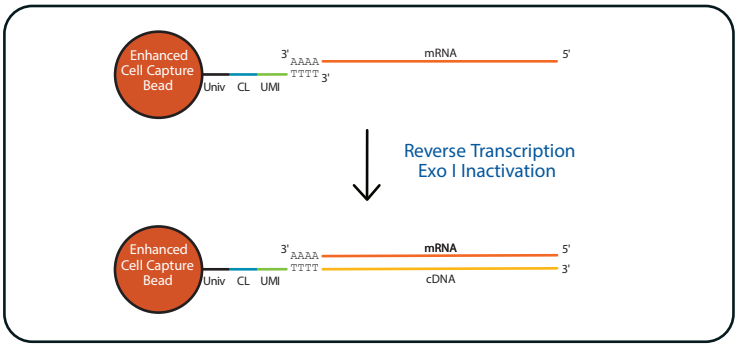
STOP!

Note: If profiling TCR/BCR, DO NOT proceed to the "Performing reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads" section.

Instead, perform reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads according to the instructions on the TCR/BCR protocols in the BD Single-Cell Multiomics Resource Library at scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library.

Performing reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads

Prepare the reverse transcription mix, wash the BD Rhapsody™ Enhanced Cell Capture Beads, and then perform reverse transcription on the beads with captured polyadenylated targets.



Univ: universal oligo; CL: cell label; UMI: Unique Molecular Identifier.

Best practices

- Prepare the cDNA mix in the pre-amplification workspace.
- Start reverse transcription within 30 minutes after washing retrieved beads with Bead Wash Buffer.

Before you begin

- Obtain the 5-mL LoBind® tube of retrieved beads. See Retrieving the BD Rhapsody™ Enhanced Cell Capture Beads from the cartridge on page 31.
- Ensure that the SmartBlock™ Thermoblock 1.5-mL or equivalent is installed on the thermomixer and is set to 37 °C for 20 minutes.

Performing reverse transcription

Note: When working with the BD Rhapsody™ Enhanced Cell Capture Beads use only low-retention tips and LoBind® tubes.

Note: Limit preparation of mixes to less than or equal to 20% overage.

Note: Prepare the cDNA mix on ice.

1. Ensure that the SmartBlock Thermoblock for ThermoMixer® C is at 37 °C.
2. In the pre-amplification workspace, into a new 1.5-mL LoBind® tube that is on ice, pipet the components in the following order to prepare the cDNA mix.

cDNA mix

Component	1 library (µL)	1 library + 20% overage (µL)
RT Buffer	40.0	48.0
dNTP	20.0	24.0
RT 0.1 M DTT	10.0	12.0
Bead RT/PCR Enhancer	12.0	14.4
RNase Inhibitor	10.0	12.0
Reverse Transcriptase	10.0	12.0
Nuclease-Free Water	98.0	117.6
Total	**200.0**	**240.0**

3. Gently vortex and centrifuge the mix, and then place it back on ice.
4. Place the tube of washed beads (see Washing the BD Rhapsody™ Enhanced Cell Capture Beads on page 32) on the 1.5-mL tube magnet for up to 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
5. Use a low-retention tip to pipet 200 µL of the cDNA mix to resuspend the beads. Gently mix the suspension by pipette only. Do not vortex.

   Note: Prepared cDNA mix with beads should be kept on ice until the suspension is transferred in the next step.

6. Transfer the bead suspension to a new 1.5-mL LoBind® tube.
7. Incubate the suspension on the thermomixer at 1,200 rpm and 37 °C for 20 minutes.

   Note: Shaking is critical for this incubation.

8. After incubation, place the tube on ice.

Treating the sample with Exonuclease I

Before you begin

- Ensure that the SmartBlock Thermoblock 1.5-mL or equivalent is installed on the thermomixer and is set to 37 °C and 30 minutes.
- Set a second thermomixer to 80 °C.

Exonuclease I inactivation temperatures above 80 °C can result in the loss of AbSeq molecules, thus a heat block should not be used for this step. If only one thermomixer is available, allow it to equilibrate to 80 °C before starting the inactivation incubation.

Preparing the Exonuclease I mix

Note: When working with BD Rhapsody™ Enhanced Cell Capture Beads, use only low-retention tips and LoBind tubes.

Note: Limit preparation of mixes to ≤20% overage.

Note: Prepare the Exonuclease I mix on ice.

1. In the pre-amplification workspace, prepare the Exonuclease I mix in a new 1.5-mL LoBind® tube that is on ice by adding the components in the following order:

Exonuclease I mix

Component	1 library (µL)	1 library + 20% overage (µL)
10X Exonuclease I Buffer	20.0	24.0
Exonuclease I	10.0	12.0
Nuclease-Free Water	170.0	204.0
Total	**200.0**	**240.0**

2. Gently vortex and centrifuge the mix, and then place it back on ice.

Treating the BD Rhapsody™ Enhanced Cell Capture Beads with Exonuclease I

1. Place the tube of beads with cDNA mix on the 1.5-mL tube magnet for ≤2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
2. Remove the tube from the magnet, and then use a low-retention tip to pipet 200 µL of Exonuclease I mix into the tube. Gently resuspend the beads by pipette only. Do not vortex.
3. Incubate the suspension on the thermomixer at 1,200 rpm and 37 °C for 30 minutes.

   Note: If only one thermomixer is available, allow it to equilibrate to 80 °C before starting the inactivation incubation. Place the samples on ice until that temperature is reached.

4. Immediately proceed to Inactivating Exonuclease I on page 36.

Inactivating Exonuclease I

1. Transfer the bead suspension with Exonuclease I to the thermomixer (no shaking) in the pre-amplification workspace at 80 °C for 20 minutes.

   Do not exceed this inactivation temperature and incubation time.

2. Place the bead suspension on ice for ~1 minute.
3. Place the tube on the 1.5-mL tube magnet until the solution is clear (within 1 minute).
4. Carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
5. Remove the tube from the magnet, and with a low-retention tip, pipet 200 µL of cold Bead Resuspension Buffer to gently resuspend the beads. Do not vortex.

   Stopping point: The Exonuclease I-treated beads can be stored at 2–8 °C for up to and including 3 months.

6. Proceed to library preparation.

7. Troubleshooting

Perform troubleshooting when the multiplet rate is higher than expected, sequencing quality is consistently lower, or noise levels as measured by sequencing are consistently higher than expected.

Note: Perform troubleshooting by microscopy first before proceeding to cartridge preparation troubleshooting.

Troubleshooting by microscopy: introduction

Troubleshooting by microscopy is an opportunity to compare your samples in the cartridge to example images in order to identify specific issues with your experiment. You can also use microscopy to determine whether metrics obtained during the cartridge workflow steps are within acceptable range.

Note: The calculations that you can perform in troubleshooting for cell loading, bead loading, and cell multiplet rate are estimates based on extrapolations of limited sample size. The calculations might differ from the sequencing results.

Troubleshooting by microscopy requires that you stain cells with viability stains, Calcein AM and Propidium Iodide, before loading the cell sample in the BD Rhapsody™ Cartridge. To view cell viability, brightfield microscopy is required and fluorescence microscopy is recommended (excitation/emission: 494 nm/517 nm and 533 nm/617 nm).

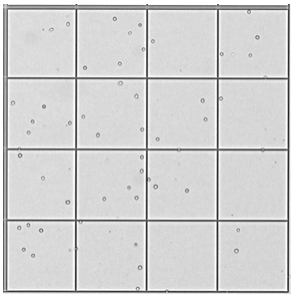
Check the BD Rhapsody™ Cartridge under a microscope after cell loading, bead loading, second bead wash, and retrieval steps. If possible, take bright-field and/or fluorescence microscopic images at each step.

After having identified specific issues with cartridge loading by microscopy, proceed to the tables of observations for additional recommended solutions. See Cartridge preparation troubleshooting on page 48.

Troubleshooting by microscopy: cell preparation of single and multiplexed samples

Obtain an image of your hemocytometer after loading it with cells for counting, and compare the image to the provided example image.

Note: Example brightfield image of a 1 × 1 mm^2 square for cell counting. Count four 1 × 1 mm^2 squares for a total of ≥100 cells.

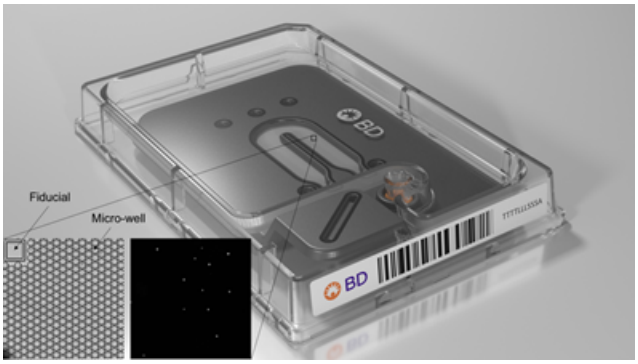


Troubleshooting by microscopy: cartridge inspection after Cell Load

This section describes how to image stained cells in order to calculate cell viability and the total number of cells captured in the cartridge. If the calculated cell viability is substantially lower after cell load than it was during cell preparation, this indicates that the health of cells is declining. Proceed with the BD Rhapsody™ workflow at your own risk.

Estimate cell viability and the total number of cells captured

1. After cell loading, incubate the cartridge at room temperature (15–25 °C) for the entire 15 minutes before imaging the cartridge.
2. Note the location of the image within the microwell array by recording the location of the fiducial (post), in order to image the cartridge at the same location for Cell Load, Bead Load, second Bead Wash, and Bead Retrieval steps in the BD Rhapsody™ workflow.



3. Image the BD Rhapsody™ Cartridge under a microscope taking brightfield and fluorescence images.
4. In the field of view under the microscope, count and record the number of:
   - Calcein AM positive cells
   - Propidium Iodide positive cells
   - Wells in the field of view

   Note: If the count of Calcein AM positive cells is <100, count cells in another field of view until the Calcein AM count is ≥100.
5. Estimate cell viability and the total number of cells captured in the cartridge:

   Viability = No. Calcein AM positive cells/(No. Calcein AM positive cells + no. of PI positive cells)

   Total no. cells captured = cells/well x 221,891

Example

In a field of view of the cartridge wells, which is arbitrarily called Region 1 [see Region 1: 26 cells on page 39] 26 cells are counted in 225 wells (15 rows × 15 columns of microwells). To count a total of ≥100 cells, an additional four regions are counted [see Region 2: 14 cells on page 40 through Region 5: 26 cells on page 41]:

Region	Cell count	No. of wells
1	26	225
2	14	225
3	17	225
4	22	225
5	26	225
Total	**105**	**1,125**

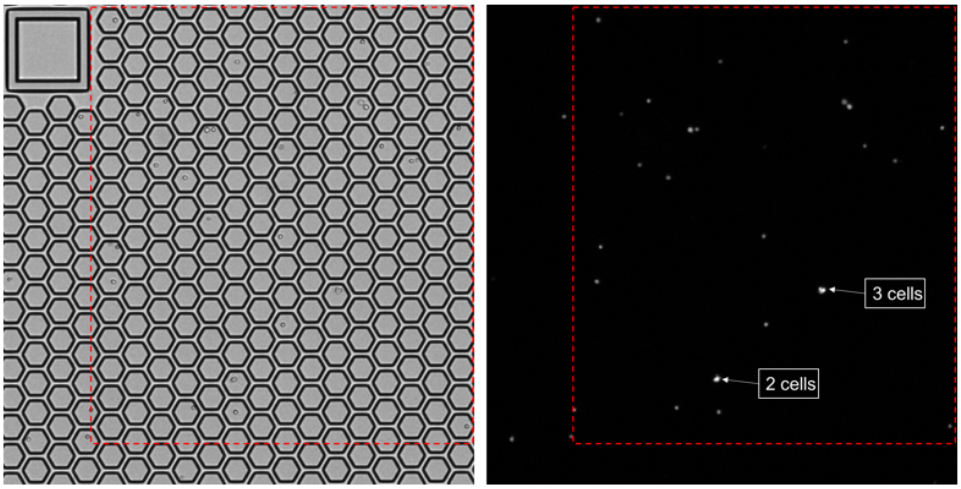
Therefore, accounting for five regions:

Cells/well = 105/1,125 = 0.0933

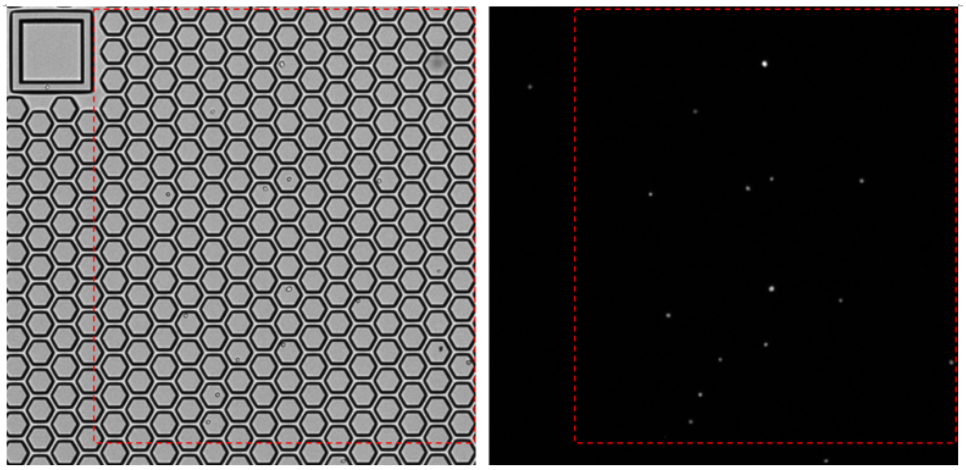
Total no. cells captured in the cartridge = 0.0933 x 221,891

Total no. cells captured in the cartridge = 20,702

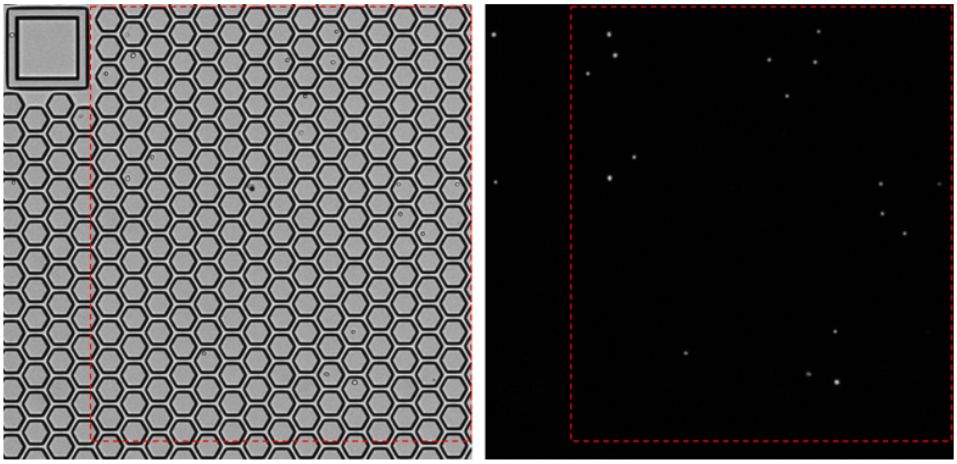
Region 1: 26 cells



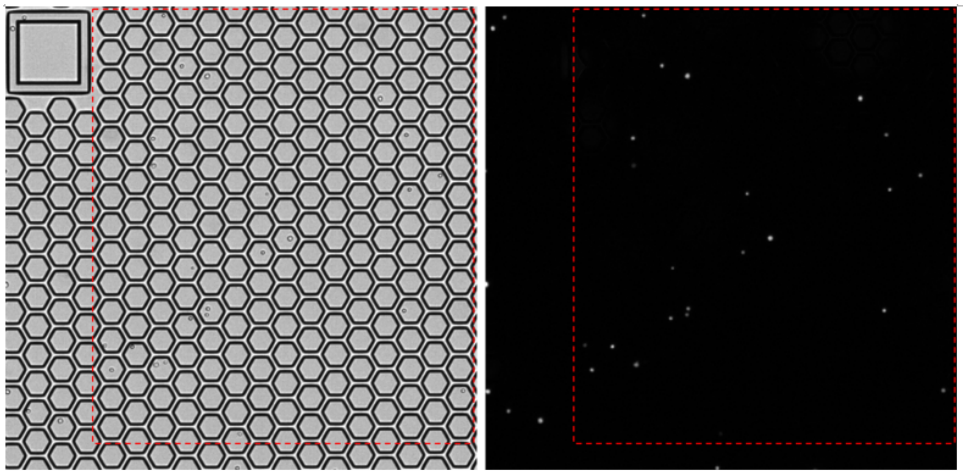
Region 2: 14 cells



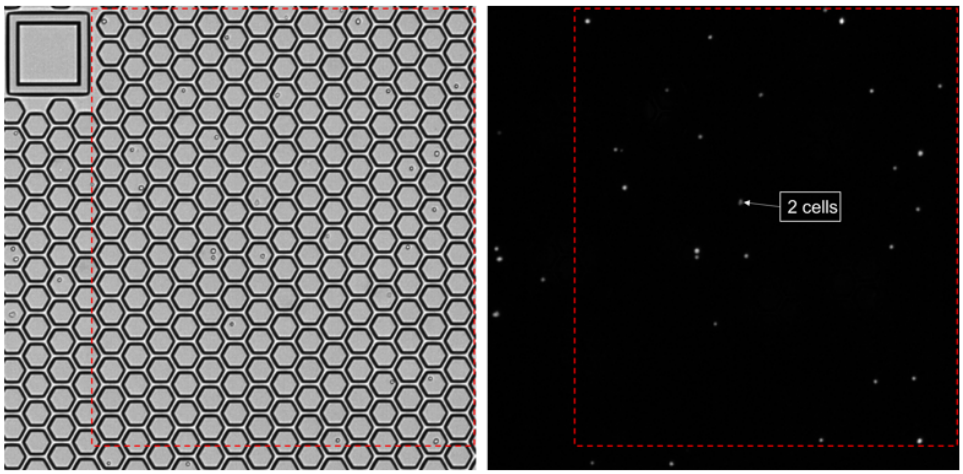
Region 3: 17 cells



Region 4: 22 cells



Region 5: 26 cells



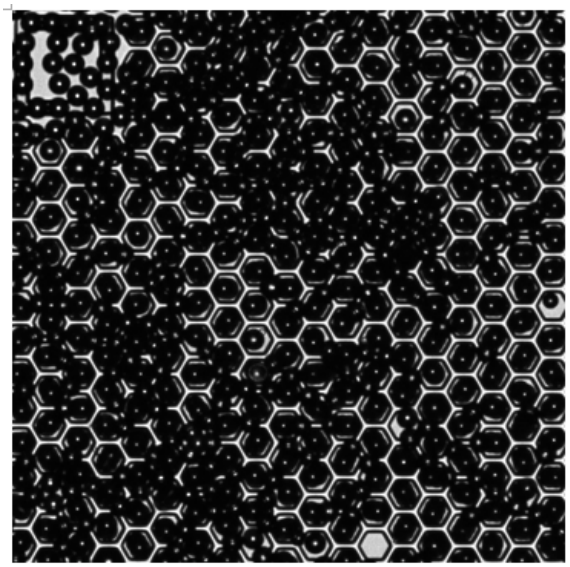
Troubleshooting by microscopy: cartridge inspection after Bead Load

After bead loading is complete and the cartridge has incubated at room temperature (15–25 °C) for an entire 3 minutes, image the BD Rhapsody™ Cartridge under a microscope. Counts of fluorescent cells might be impacted by the presence of beads; therefore, fluorescent cell counts do not conclusively infer cell loss at this step. However, comparative losses of beads in brightfield images might indicate insufficient bead loading.

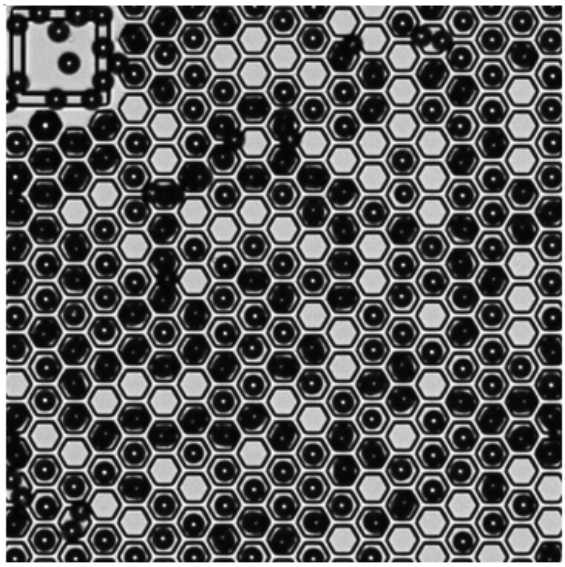
Example images after Bead Load

Obtain images of your cartridge after Bead Load, and compare them to the provided example images.

Acceptable loading of BD Rhapsody™ Enhanced Cell Capture Beads after bead loading



Insufficient loading of BD Rhapsody™ Enhanced Cell Capture Beads after bead loading



Troubleshooting by microscopy: cartridge inspection after Bead Wash

After the second bead wash, you can calculate the bead loading efficiency, cell multiplet rate, and number of beads captured in the cartridge. These metrics are important to determine the efficiency of cartridge loading.

Estimate the bead loading efficiency and number of captured cells on BD Rhapsody™ Enhanced Cell Capture Beads

1. After the Bead Wash step is complete, image the BD Rhapsody™ Cartridge under a microscope. Brightfield microscopy is required, and fluorescence microscopy is recommended.
2. Note the location of the image within the microwell array by recording the location of the fiducial (post) in order to image the cartridge at the same location.
3. Calculate the bead loading efficiency:
   a. Count the number of beads in ≥200 wells.
   b. Calculate:

      Bead loading efficiency = No. wells containing one bead / no. wells

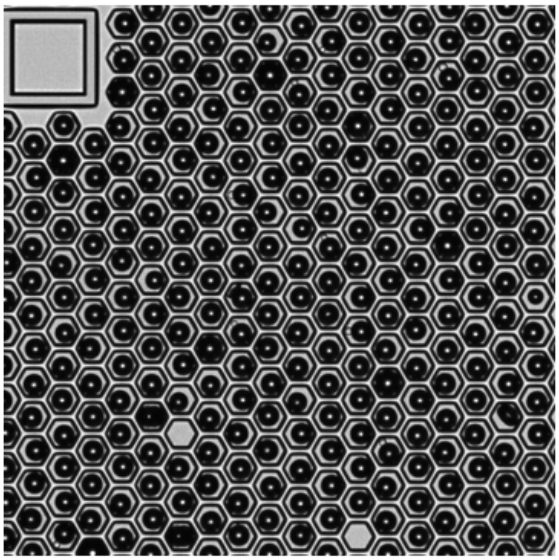
4. Estimate the total number of cells captured in the cartridge. See Estimate cell viability and the total number of cells captured on page 38. You will use the total number of cells captured in the cartridge in **step 6**.
5. Use the concentration of cell suspension at Cell Load to find the estimated multiplet rate. See the multiplet rate table in Preparing a single-cell suspension for cartridge loading on page 25. You will use the estimated multiplet rate in **step 6**.
6. Estimate the number of captured cells on BD Rhapsody™ Enhanced Cell Capture Beads:

   No. captured cells on beads = (Bead loading eff x no. cells captured x (100 – est multiplet rate) / 100)

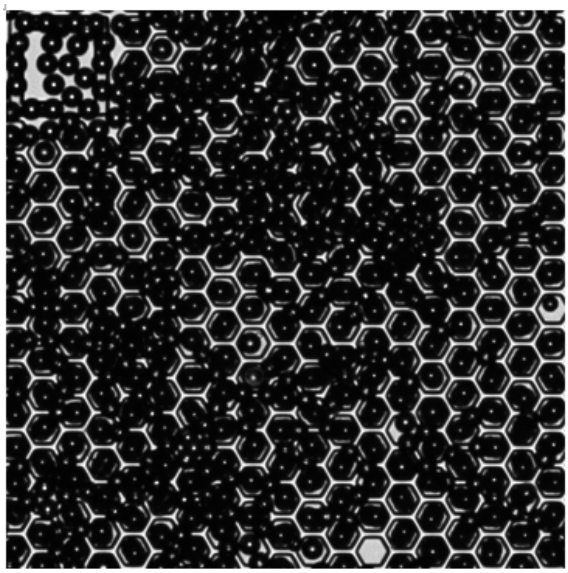
Example images after Bead Wash

Obtain images of your cartridge after Bead Wash, and compare them to the provided example images.

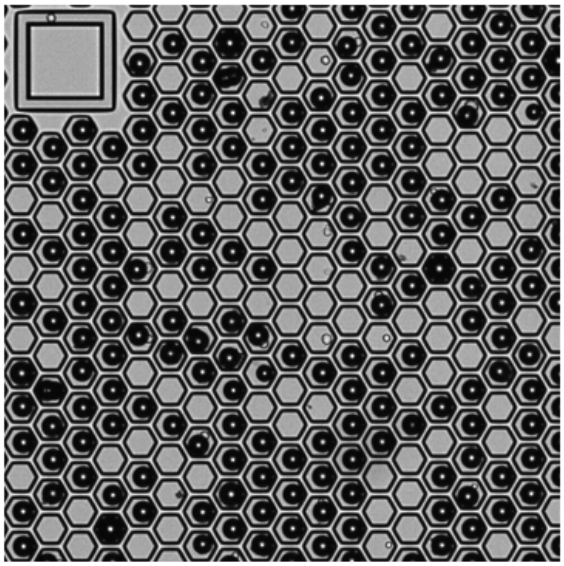
Acceptable bead number after the second bead wash



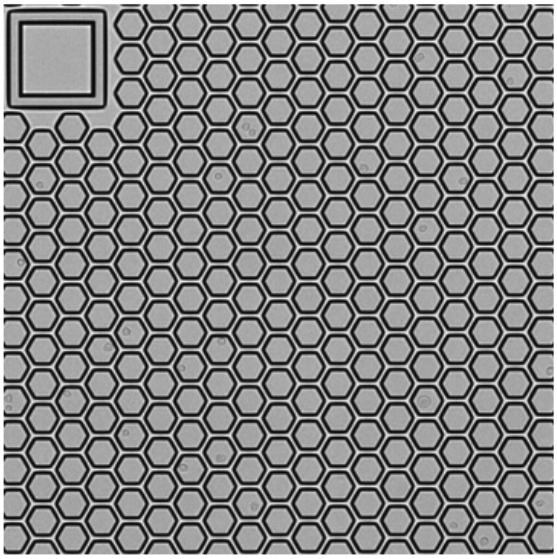
Insufficient removal of excess BD Rhapsody™ Enhanced Cell Capture Beads



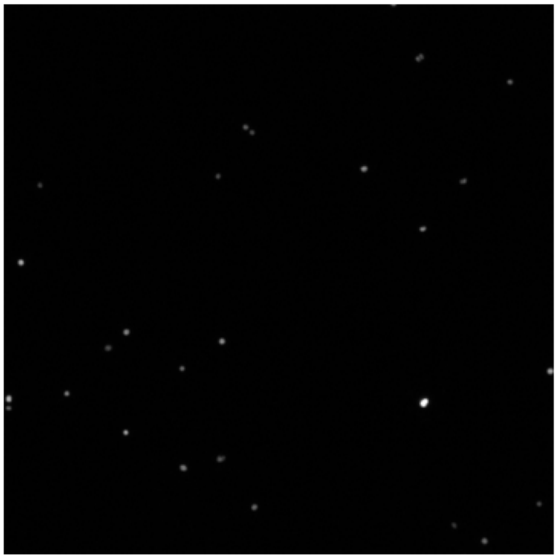
Significant loss of BD Rhapsody™ Enhanced Cell Capture Beads resulting in empty microwells after the second bead wash



Significant cell loss after second bead wash: Images after cell loading

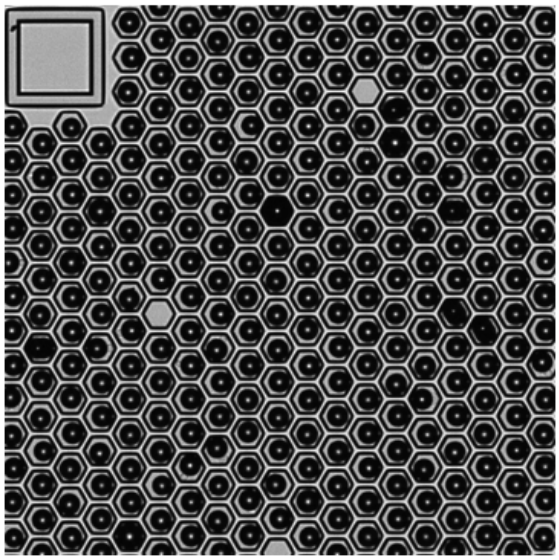


Brightfield



Fluorescent

Significant cell loss after the second bead wash: Images after the second bead wash



Brightfield

Fluorescent

Troubleshooting by microscopy: cartridge inspection after Bead Retrieval

After bead retrieval, determine how many beads remain in the cartridge. Beads that are not retrieved might contribute to loss of cells during the BD Rhapsody™ workflow. A larger than normal percentage of beads remaining in wells (% beads/well >10) after retrieval might partially account for a lower number of cells in sequencing than expected.

Estimate the number of remaining beads after Bead Retrieval

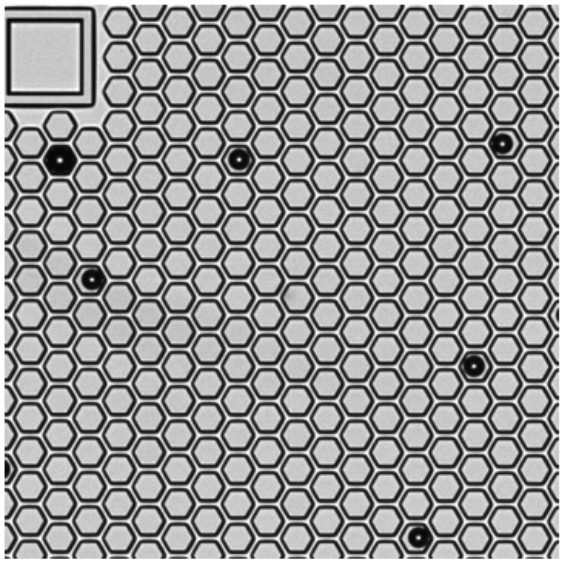
1. After bead retrieval is complete, image the BD Rhapsody™ Cartridge under a brightfield microscope. Quantities of beads observed in brightfield images might indicate insufficient bead retrieval.
2. Calculate the remaining beads in the cartridge after bead retrieval:
   a. Count the number of beads in ≥200 wells.
   b. Calculate:

      Percent remaining beads = No. wells containing one bead / (No. wells)

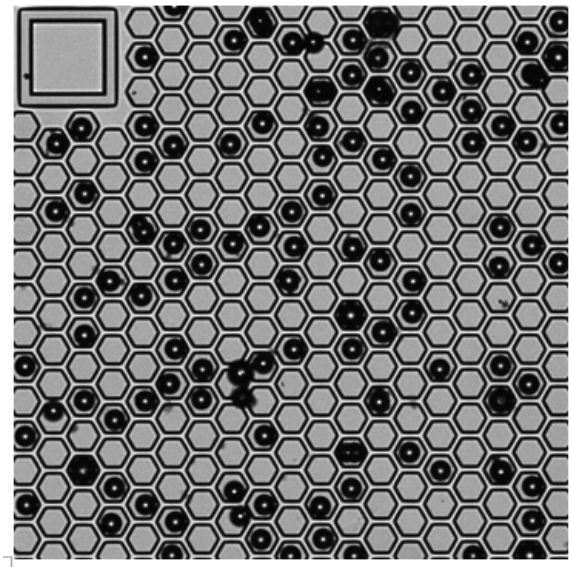
Example images after Bead Retrieval

Obtain images of your cartridge after Bead Retrieval, and compare them to the provided example images.

Acceptable retrieval of BD Rhapsody™ Enhanced Cell Capture Beads



Poor retrieval of BD Rhapsody™ Enhanced Cell Capture Beads



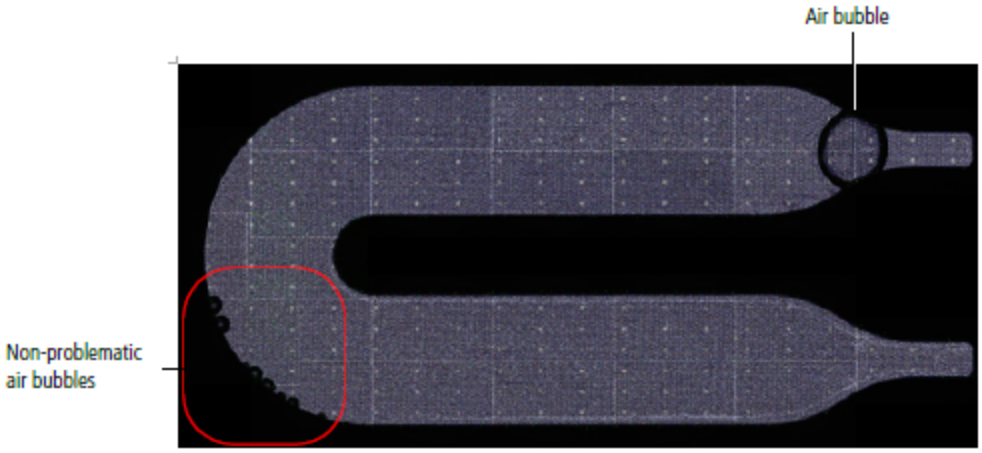
Troubleshooting by microscopy: BD Rhapsody™ Cartridge handling

Under the microscope, distinguish air bubbles in the cartridge that can decrease bead capture from non-problematic air bubbles.

Example image of the BD Rhapsody™ Cartridge

Obtain images of your cartridge after each workflow step, and compare each image to the provided example image.

Air bubble in cartridge after Bead Wash. In this brightfield example, the yield of final retrieved BD Rhapsody™ Enhanced Cell Capture Beads would decrease by ~3%. Check the cartridge after each workflow step.



Air bubbles in a cartridge are rare. If there is an air bubble, proceed with the experiment. Follow the user guide to prevent air bubbles from entering the cartridge.

Cartridge preparation troubleshooting

First, perform troubleshooting by microscopy to identify issues with the cartridge workflow before using the cartridge preparation troubleshooting tables in this section. See Troubleshooting by microscopy: introduction on page 37. Next, match identified issues with the listed observations in the table and follow the recommended solutions.

Cartridge preparation troubleshooting

Observation	Possible causes	Recommended solutions
After Cell Load		
Cell viability is significantly lower than the viability observed during cell preparation.	Suboptimal sample quality and/or sample handling.	• Remove dead cells by standard procedures. • Keep cells on ice. • Reduce the time from cell preparation to cell loading in the BD Rhapsody™ Cartridge. • Check the viability dye staining to ensure it did not fade. If cells were not stained at 37 °C properly, fluorescence could bleach upon light exposure during microscopy scanning.
Estimated cells captured is significantly higher than the estimated cells loaded.	• Incorrect cell concentration. • Incorrect dilution.	• Recount the cells or use a different counting method. • Recounting and recalculating would apply to a new cartridge. Proceed at own risk with current cartridge. • Recalculate the cell dilution.
	Improper cell counting.	• Follow best practices for cell counting. See Best practices for cell handling and cell counting on page 21.
Estimated cells captured is significantly lower than the estimated cells loaded.	• Incorrect cell concentration • Incorrect dilution	• Recount the cells or use a different counting method. • Recounting and recalculating would apply to a new cartridge. Proceed at own risk with current cartridge. • Recalculate the cell dilution.
	Improper cell counting	• Follow best practices for cell counting. See Best practices for cell handling and cell counting on page 21.
	Cell diameter >20 µm	• The percentage of cells recovered in sequencing declines for cells >20 µm in diameter. Load additional cells to offset the losses observed.
After Bead Load		
Too few beads loaded	Incorrect preparation of BD Rhapsody™ Enhanced Cell Capture Beads.	• Confirm underloading of beads with example image. See Acceptable loading of BD Rhapsody™ Enhanced Cell Capture Beads after bead loading on page 41. • Pellet BD Rhapsody™ Enhanced Cell Capture Beads and resuspend them in Sample Buffer. • Thoroughly resuspend beads before loading into cartridge.
	Wrong pipette mode.	• Use the **Bead Load** mode on the BD Rhapsody™ P1200M pipette.

Cartridge preparation troubleshooting

Observation	Possible causes	Recommended solutions
After Bead Wash		
Observed cell multiplets are significantly higher than the expected multiplet rate.	Cell samples that tend to clump.	• Filter the cell suspension through an appropriately sized cell strainer multiple times to remove clumps and doublets.
Insufficient removal of excess BD Rhapsody™ Enhanced Cell Capture Beads.	Bead wash steps missed.	• Confirm excess beads with images. See Acceptable bead number after the second bead wash on page 43. • Repeat the two bead wash steps after shaking the cartridge on the plate shaker.
	Wrong pipette mode.	• Use the Wash mode on the BD Rhapsody™ P1200M pipette for the two bead.
Estimated bead load efficiency is <80%.	Cartridge shaking step skipped.	• Confirm loss of cells with images. See Acceptable bead number after the second bead wash on page 43 and Significant loss of BD Rhapsody™ Enhanced Cell Capture Beads resulting in empty microwells after the second bead wash on page 44. • Repeat the experiment with a new cartridge. Ensure that the cartridge is shaken on the plate shaker before the two bead wash steps.
	Wrong plate shaker settings.	• Repeat the experiment with a new cartridge. Use the Wash mode on the BD Rhapsody™ P1200M pipette for the two bead wash steps.
	Wrong pipette mode.	• Repeat the experiment with a new cartridge. Use the Wash mode on the BD Rhapsody™ P1200M pipette for the two bead wash steps.
Significant loss of cells at Bead Wash.	Cell viability low.	• Confirm loss of cells with images. See After Bead Retrieval on page 50 and Significant cell loss after the second bead wash: Images after the second bead wash on page 45. • Try to ensure that cell viability is ≥50%.
	Incorrect removal of buffer from the cartridge through pipette aspiration.	• Only use the pipette in the correct mode to dispense air into the cartridge to remove buffer.
	Wrong pipette mode.	• Use the correct pipette mode at every step.
	Insufficient incubation after cell loading.	• Make sure incubation time after cell loading is 15 minutes and not less.

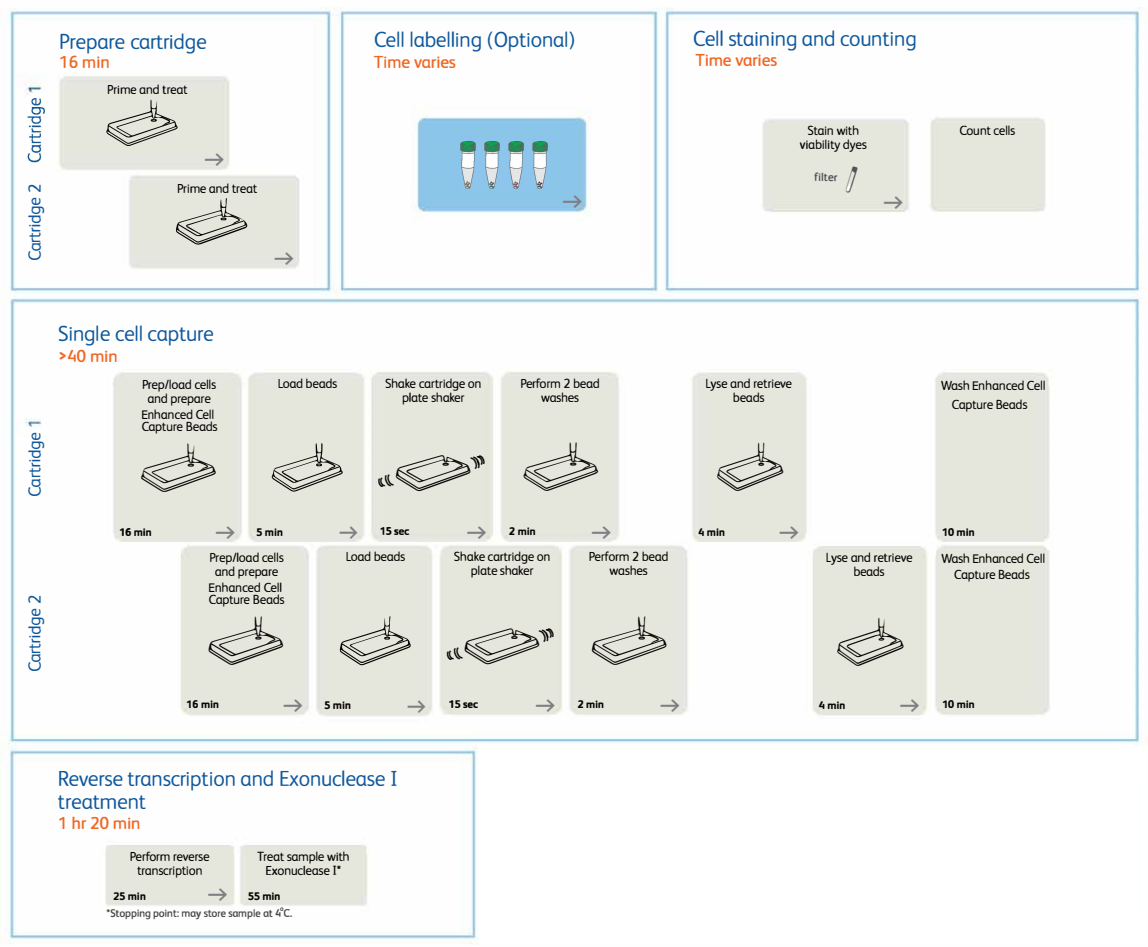
Cartridge preparation troubleshooting

Observation	Possible causes	Recommended solutions
After Bead Retrieval		
Remaining beads >10% of wells with beads.	Retrieval (top) magnet not in down position during retrieval step.	• Confirm poor retrieval of beads with images. See Poor retrieval of BD Rhapsody™ Enhanced Cell Capture Beads on page 46. • Ensure that the retrieval magnet is in the down position for 30 seconds before retrieving the BD Rhapsody™ Enhanced Cell Capture Beads.
	Wrong pipette mode.	• Use the Retrieval mode on the BD Rhapsody™ P5000M pipette.
Cartridge handling		
Dropped the cartridge or hit it against an object.	Various.	• If the cartridge was dropped, We recommend using a new cartridge. • If the cartridge was struck, proceed at your own risk.
Air bubble in cartridge.	Air bubble present in pipette tip while dispensing buffer.	• Ensure that the pipette tip contains only buffer and no air bubble is trapped at the end of aspiration of buffer. • **Note:** Cells will not be lysed and beads will not be retrieved under the bubble.
	Re-used pipette tip.	• Use a new pipette tip at every pipetting step.

8. Workflow with two BD Rhapsody™ Cartridges

Workflow with two cartridges

Staggered boxes indicate staggering the start of like steps.



Best practices with a two-cartridge workflow

Reagent preparation

To prepare a master mix of sufficient volume for two cartridges, follow the volumes for two libraries plus 10% overage listed for preparing a master mix.

9. Safety

General safety and limitations

For instrument safety, see the *BD Rhapsody™ Express Instrument Safety and Limitations Guide*.

Single-Cell Multiomics technical publications are available for download from the BD® Single-Cell Multiomics Resource Library at scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library.

Chemical safety

Requirements

- Read and comprehend all safety data sheets (SDSs) by chemical manufacturers before you use, store, or handle any chemicals or hazardous materials.
- Wear personal protective equipment (gloves, safety glasses, fully enclosed shoes, lab coats) when handling chemicals.
- Do not inhale fumes from chemicals. Use adequate ventilation, and return caps to bottles immediately after use.
- Check regularly for chemical spills or leaks. Follow SDS recommendations for cleaning up spills or leaks.

Waste

The BD Rhapsody™ Express Single-Cell Analysis system has two waste types or streams. Each waste stream requires individual consideration for safe and responsible disposal:

Waste	Description
Stream 1: Waste Collection Container	• Frequency of Handling: every BD Rhapsody™ experiment. • Content: ethanol (11%), polymer micro particles (<1%), cells (trace). • Main Risk Constituent: cells (trace). • Collect and dispose of all waste in the Waste Collection Container using proper precautions and according to local safety regulations.
Stream 2: BD Rhapsody™ Cartridge	• Frequency of Handling: every BD Rhapsody™ experiment. • Content: polymer (99%), polymer micro particles (<1%), Lysis Buffer (<1%). • Main Risk Constituent(s): Lysis Buffer. • Collect and dispose of all used BD Rhapsody™ Cartridges using proper precautions and according to local safety regulations.

Physical safety

See the *BD Rhapsody™ Express Instrument Safety and Limitations Guide*.

Single-Cell Multiomics technical publications are available for download from the BD® Single-Cell Multiomics at scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library.

Instrument waste disposal

Contact BD Biosciences technical support at scomix@bdscomix.bd.com before disposing of the BD Rhapsody™ Express instrument. For more information, see Instrument technical support on page 6.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, California 95131 USA

bdbiosciences.com
scomix@bdscomix.bd.com