BD Rhapsody™ HT Xpress System

Instrument User Guide for Scanner-free workflow

23-24256(01)
2023-02

For Research Use Only

Regulatory information

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

History

Revision	Date	Change made
23-24256(01)	2023-02	Initial release.

Contents

1. Introduction

About this guide

This guide provides instructions for operating the BD Rhapsody™ HT Xpress System and supporting materials.

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™ HT Xpress System is intended for the preparation of single-cell mRNA sequencing libraries. Proprietary BD® Molecular Indexing technology is used to count individual mRNA and protein molecules.

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™ HT Xpress System.

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

For more information on the purpose of the BD Rhapsody™ HT Xpress System, see the BD Rhapsody™ HT Xpress System overview on page 9.

Restrictions

Any use of the BD Rhapsody™ HT Xpress System other than the procedures as described in this user guide or in BD Rhapsody™ protocols 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™ HT Xpress System that does not comply with the procedures described in any guide used with the BD Rhapsody™ HT Xpress 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™ HT Xpress System warranty.

Disclaimer

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

Safety symbols

For safety and limitations, see the *BD Rhapsody™ HT Xpress System 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

Before handling chemicals, read and understand the Safety Data Sheets (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

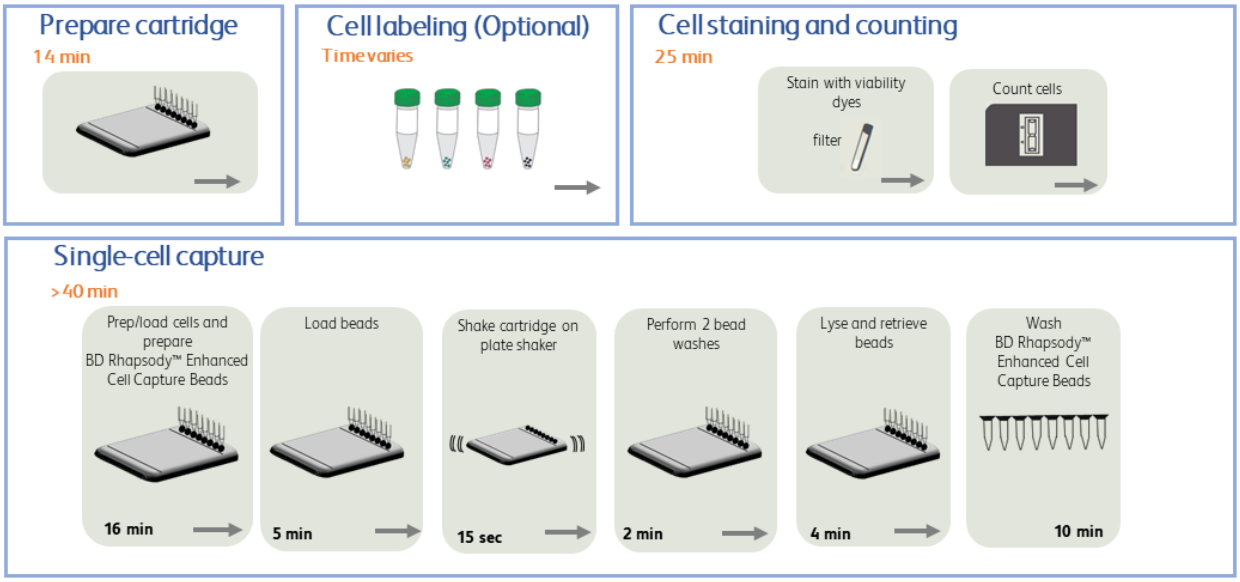
If technical assistance is required, contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com or 1.877.232.8995. You can contact technical support in Europe at help.biosciences@bd.com or +32.53.720.600.

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™ 8-Lane Cartridge workflow



Note: When using two cartridges, see Workflow with two BD Rhapsody™ 8-Lane Cartridges on page 52.

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™ HT Xpress System Site Preparation Guide*.

For installation of the BD Rhapsody™ HT Xpress System, see the *BD Rhapsody™ HT Xpress Installation and Maintenance 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.

Plate shaker settings

The following plate shakers have been validated for use on the BD Rhapsody™ HT Xpress System:

- Eppendorf ThermoMixer® C: 1,000 rpm
- Eppendorf MixMate®: 1,000 rpm

- MicroPlate Genie®: 1,600 rpm

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

Pipette settings

The BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) are provided pre-programmed for use during single-cell mRNA capture from the BD Rhapsody™ 8-Lane Cartridge. Do not change the settings but confirm pipette mode is correct before use.

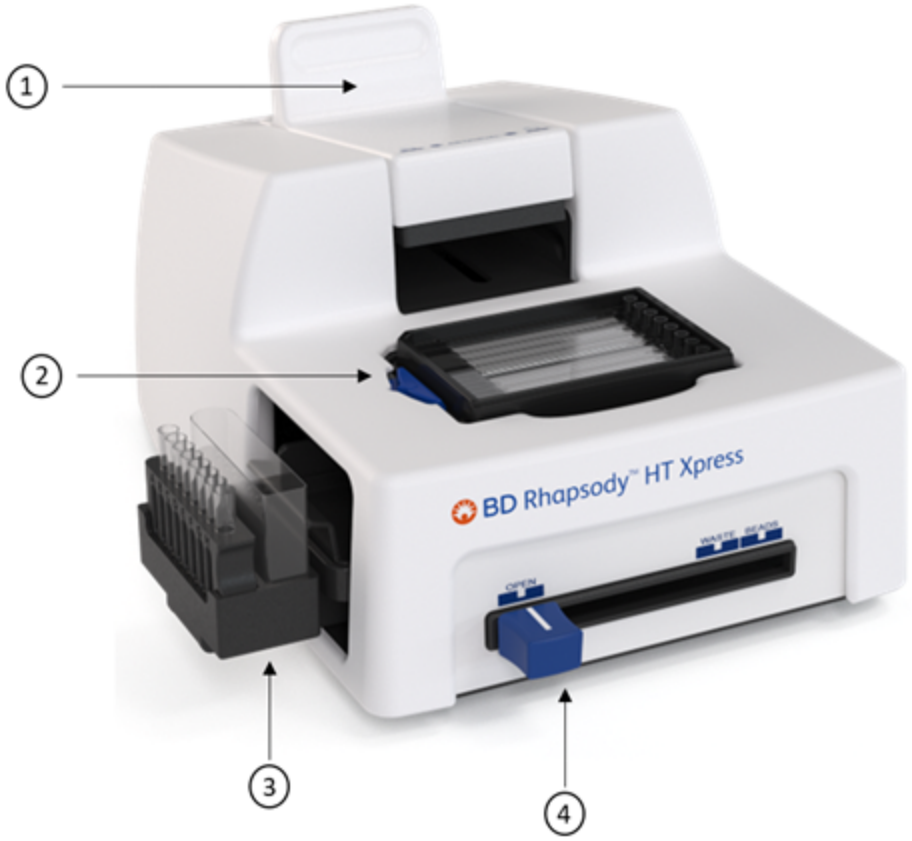
Pipette	Mode
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	EtOH/Prime
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	Prime/Wash
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	Mix
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	Load
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	Lysis
BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX)	Retrieval

BD Rhapsody™ HT Xpress System overview

The BD Rhapsody™ 8-Lane Cartridge requires the use of the BD Rhapsody™ HT Xpress System. This sample loading station is used to load reagents, cells, and BD Rhapsody™ Enhanced Cell Capture Beads into individual wells leading to lysis, capture, and retrieval of barcoded genomic and proteomic information.

For safety and limitations of the BD Rhapsody™ HT Xpress System, see the *BD Rhapsody™ HT Xpress System Safety and Limitations Guide*.

The following figure shows the main components of the BD Rhapsody™ HT Xpress System for operation. For maintenance of the BD Rhapsody™ HT Xpress System, see the *BD Rhapsody™ HT Xpress Installation and Maintenance Guide*.



No.	Component
1	Retrieval (top) magnet. Magnet shown in neutral position: No magnets applied to the BD Rhapsody™ 8-Lane Cartridge.
2	Cartridge tray to insert the BD Rhapsody™ 8-Lane Cartridge.
3	Bead retrieval tube holder (left) and waste collection container (right).
4	Front slider to position: Waste collection container and bead retrieval tube access (OPEN), waste collection container (WASTE), and bead retrieval tube (BEADS).

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™ HT Xpress Installation and Maintenance Guide*.
- 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.
- 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.
- 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 23.

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™ HT Xpress System

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

BD Rhapsody™ 8-Lane 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 airtight seal with the BD Rhapsody™ P8xP1200µL Pipette – HTX, (or BD Rhapsody™ P1200µL Pipette – HTX) see Pipetting instructions on page 17.
- Hold the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) with one hand and firmly seal pipette tips on to the inlets of the lanes in the cartridge.
- Cells need to be prepared as close to cell loading in the cartridge as possible. Begin cell preparation after the prime and 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™ 8-Lane Cartridge
- BD Rhapsody™ cDNA Kit

The cartridge-related kits listed are included in each of the following 8-pack products that also contain an amplification kit.

- Targeted mRNA and AbSeq Amplification Kit - 8 pack
- Whole Transcriptome Analysis (WTA) Amplification Kit - 8 pack
- TCR/BCR Amplification Kit, Human - 8 pack
- TCR/BCR Amplification Kit, Mouse - 8 pack

Required reagents

Required reagents

Material	Supplier	Catalog no.
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
BD Rhapsody™ 8-Lane Cartridge	BD Biosciences	666262
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
Dimethyl sulfoxide (DMSO)	Major supplier	–
Propidium iodide, 1mg/mL in water[a]	Thermo Fisher Scientific	P3566
Trypan blue stain, 0.4%	Thermo Fisher Scientific	15250061
70% ethyl alcohol or 70% isopropyl alcohol[b]	Major supplier	–

a. Protect Calcein AM from light. Avoid multiple freeze-thaw cycles of Calcein AM. See manufacturer's storage recommendations.

b. To clean the BD Rhapsody™ HT Xpress System and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ HT Xpress Installation and Maintenance Guide*. Instead of 70% alcohol, 10% (v/v) bleach can be used.

Required consumables

Required consumables

Material	Supplier	Catalog no.
Gilson™ PIPETMAN™ Tipack™ filter tips, 100-1200 µL for BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) Or, ZAP™ SLIK 1000 µL low retention aerosol filter pipette tips for BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) (alternative)	Thermo Fisher Scientific Labcon	F171803G 1177-965-008-9
60 mL reagent reservoir self-standing[a]	BD Biosciences	666626
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 10 mL	VistaLab	3054-1012 3054-1013
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 25 mL	VistaLab	3054-1002 3054-1003
Falcon® tube with cell strainer cap	Corning	352235
Corning® 96-well polypropylene cluster tubes, 8-tube strip format, sterile[b]	Corning	4413
DNA LoBind® tubes, 1.5-mL	Eppendorf	30108051
Low-retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
Deep 96-well 2 mL polypropylene plate	Major supplier	–
Pre-moistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol	Major supplier	–
Lint-free wipes	Major supplier	–

a. Waste collection container for the BD Rhapsody™ HT Xpress System.
b. Bead retrieval tubes for the BD Rhapsody™ HT Xpress System.

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™ HT Xpress[a]	BD Biosciences	666730
BD Rhapsody™ P1200µL Pipette – HTX[a]	BD Biosciences	500066148
BD Rhapsody™ P8xP1200µL Pipette – HTX[a]	BD Biosciences	666718
Cell counter	Major supplier	–
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Centrifuge and rotor with adapters for 5-mL Falcon tubes and 15-mL tubes	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
Incubator at 37 °C	Major supplier	–
Pipettes (P10, P20, P200, P1000)	Major supplier	–
Vortexer	Major supplier	–
Digital timer	Major supplier	–
6-Tube magnetic separation rack for 1.5-mL tubes Or, 12-Tube magnetic separation rack Or, Invitrogen™ DynaMag™-2 magnet	New England Biolabs New England Biolabs Thermo Fisher Scientific	S1506S S1509S 12321D

a. Part of the BD Rhapsody™ HT Xpress System. Items can be ordered separately.

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 BD Rhapsody™ 8-Lane Cartridge

Before you begin

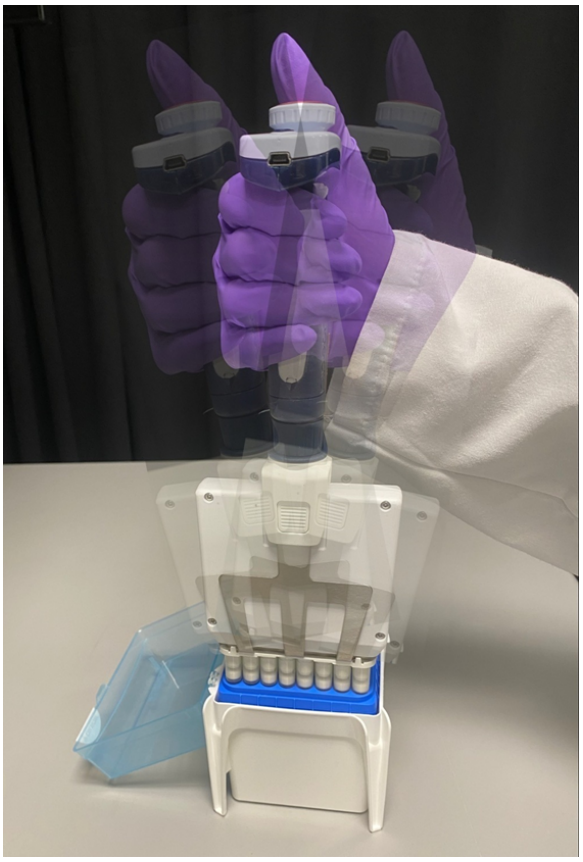
Visually inspect the Lysis Buffer for any precipitation. If precipitation is present, incubate the Lysis Buffer at room temperature (15–25 °C) for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, place the Lysis Buffer on ice.

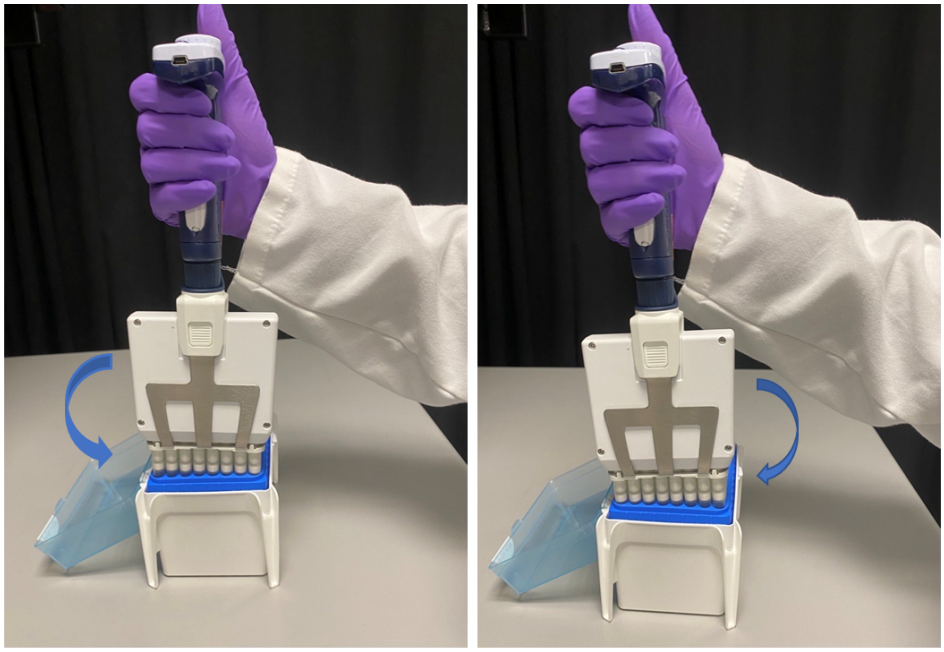
If cell preparation takes 4 hours or longer, begin preparing cells before cartridge preparation.

- 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
  - BD Rhapsody™ Enhanced Cell Capture Beads
  - 1 M DTT
  - Lysis Buffer
- Thaw Calcein AM. Once at room temperature (15–25 °C), resuspend Calcein AM in 503.0 µL of DMSO for a final stock concentration of 2 mM. Follow the manufacturer's storage recommendations, and protect it from light.
- Thaw reagents (not enzymes) in the BD Rhapsody™ cDNA Kit at room temperature (15–25 °C), and then place on ice. Keep enzymes at –25 °C to –15 °C, see *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol*.
- After opening the cartridge packet, ensure that you prepare only the lanes you intend to use in the cartridge just before the start of the experiment.
- Review pipette settings and operation. See Pipette settings on page 8.
- For the use of two cartridges, see Workflow with two BD Rhapsody™ 8-Lane Cartridges on page 52.

Pipetting instructions

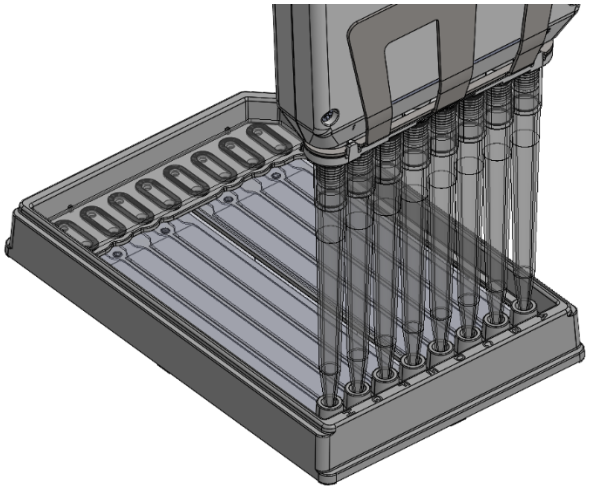
- For the BD Rhapsody™ P1200µL Pipette – HTX, push the tip holder into the tip using a slight twisting motion to ensure a firm and airtight seal.
- The battery is charged in the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX). Charging may only be carried out using the charging adapter supplied or the charging stand with the original power unit.
- To ensure an airtight seal with the BD Rhapsody™ P8xP1200µL Pipette – HTX, the Rocky Rack available in the Gilson Towerpacks is recommended. It is a dome shaped part of the pack which holds the tips.
- Hold the pipette with one hand and move it at an angle on either side of the rack back and forth a few times. This gives a tight seal to the pipette tips on the shaft of all the channels without the need of pressure or touching the tips after (see the following image).





Tilt/Rock the pipette toward either side of the pipette tips (placed in a column as shown above) a couple of times to ensure a proper fit.

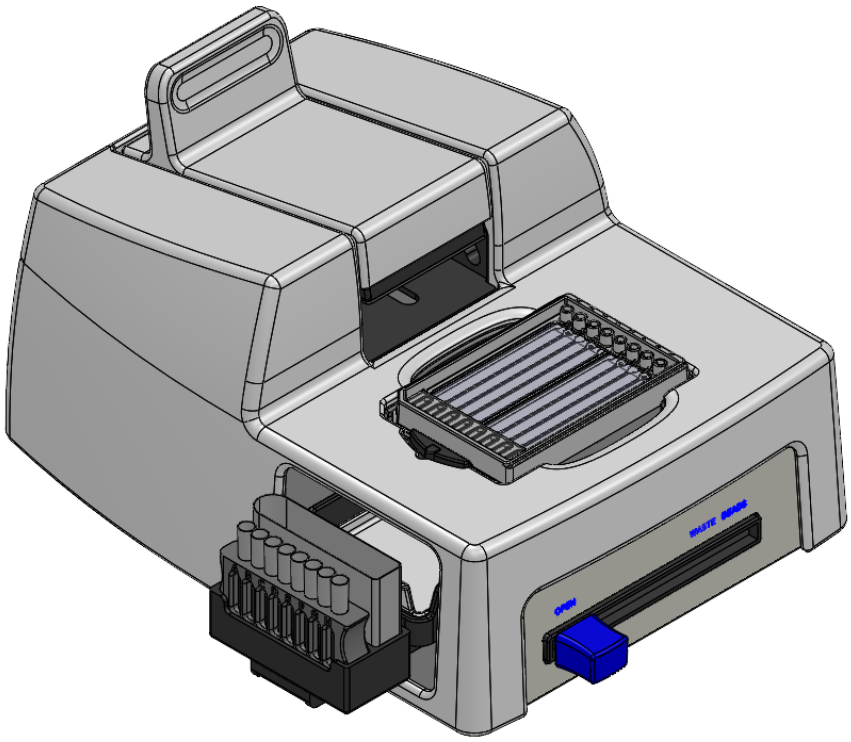
- To ensure accuracy of volumes in all the channels, pre-rinse the tip by aspirating and dispensing the first volume of liquid. This will prevent a thin layer forming inside tip while using protein and organic solvents.
- Immerse the tips completely in the liquid and aspirate the desired volume by pressing the push button. Wait for a couple of seconds before withdrawing the tips. The excess liquid can be wiped with a medical wipe.
- While removing pipette from the reservoir, draw the tip along the inside surface of the vessel.
- Before dispensing, carefully remove droplets from the outside of the tip by touching off the side of the reservoir.
- When dispensing, ensure that the pipette tips are seated perpendicular to the BD Rhapsody™ 8-Lane Cartridge. Align the pipette tips based on the number of lanes used with the inlet holes of the gasket, and then press down on the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to seal the pipette tip against the gasket and avoid leaks.
- Press the push button and wait for a few seconds before releasing it to dispense the liquid.



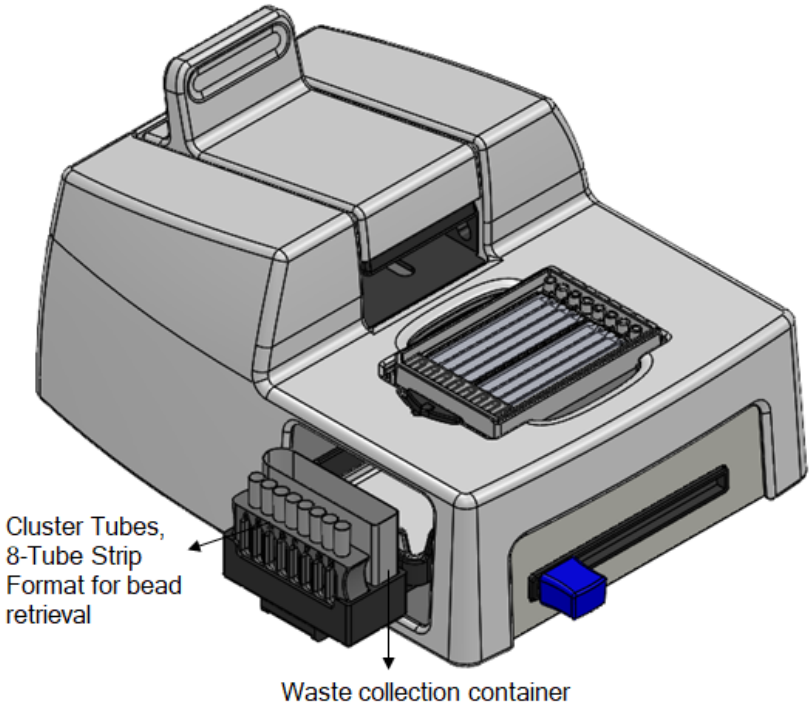
- Avoid introducing bubbles while pipetting into the BD Rhapsody™ 8-Lane Cartridge.
- Change pipette tips before every pipetting step.
- Always use low-retention filtered pipette tips and LoBind tubes.

Setting-up BD Rhapsody™ HT Xpress System

1. Make sure the Retrieval (top) magnet is away from the cartridge tray.
2. Move the front slider to **OPEN**:



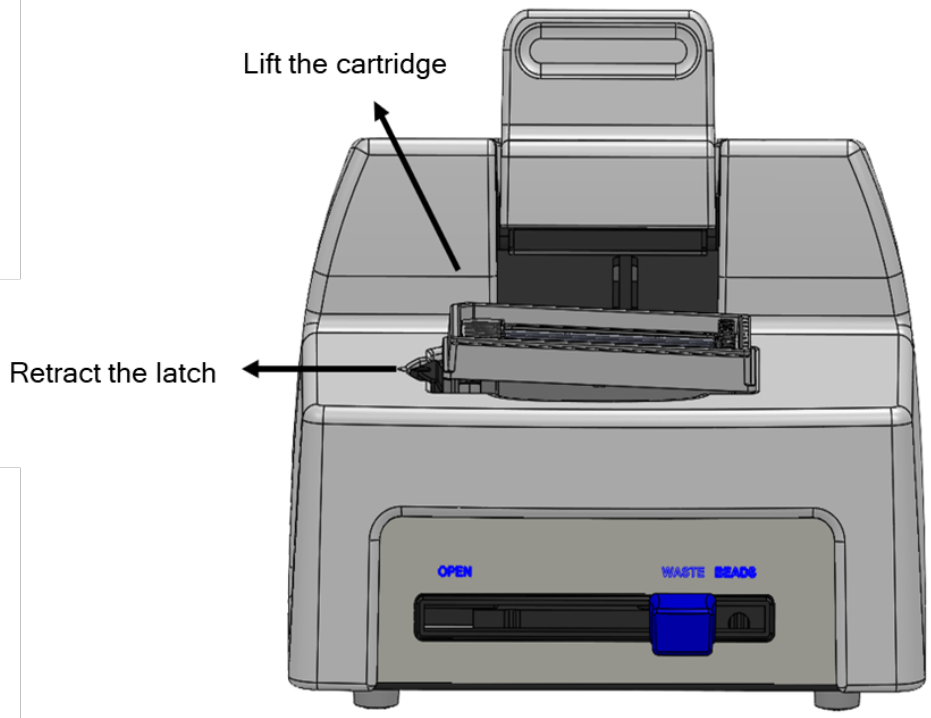
3. Remove the cap of a waste collection container. Insert both the waste collection container and the cluster tubes for bead retrieval into the appropriate slots in the drawer. When using partial lanes, use the corresponding number of tubes for sample retrieval.



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

5. Push the cartridge into the far end of the tray on the left 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 on the right side.

Note: To remove the cartridge from the BD Rhapsody™ HT Xpress System, retract the latch toward the outside of the BD Rhapsody™ HT Xpress System with one hand and lift the cartridge off the top plate with the other.



Priming BD Rhapsody™ 8-Lane Cartridge

- Keep the foil pouch and desiccant to store a partially used cartridge.
- Carefully peel off the seal on the cartridge inlet for each lane being used.
- Set the instrument sliders in the following positions.

BD Rhapsody™ HT Xpress System slider	Position
Front Slider	Waste
Retrieval Slider	Inactive (Back)

Notes:

- EtOH priming of the cartridge followed by air purge provides full coverage of the array during the Prime/Wash step (Step 2 in the following table).
- Random bubbles (<3 mm diameter in size) that occur during the Priming steps does not affect cartridge performance.

- If bubbles >3 mm in size are observed, aspirate and dispense air using the Prime/Wash mode and repeat Step 1 with 100% ethyl alcohol. Only do this in the Priming steps.
- Uneven fluidic front observed on different lanes does not affect cartridge performance.
- Residual volume in the tips is expected after dispensing. Discard tips.
- It is recommended to use a P20 pipette to aspirate buffer pooling at the inlet. Aspirate at an angle to avoid accidental aspiration of buffer volume in the microwell array. Only do this in the Priming steps.
- Aliquot 100% ethyl alcohol and cartridge reagent buffers in 10-mL or 25-mL reagent reservoirs as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table:

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
100% ethyl alcohol	0.05	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cartridge Wash Buffer 1	0.76	3.50	5.25	7.00	8.75	10.50	12.25	14.00
Cartridge Wash Buffer 2	0.38	2.00	3.00	4.00	5.00	6.00	7.00	8.00

1. Load the cartridge with 50 µL of 100% Ethyl Alcohol using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **EtOH Prime** mode.
2. Load the cartridge with 380 µL of Air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
3. Load the cartridge with 380 µL of Cartridge Wash Buffer 1 using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode and leave it for 1 minute.

Treating surface of BD Rhapsody™ 8-Lane Cartridge

1. Load the cartridge with 380 µL of air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
2. Load the cartridge with 380 µL of Cartridge Wash Buffer 1 using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
3. Leave the cartridge on the tray at room temperature (15–25 °C) for 10 minutes.
4. Load the cartridge with 380 µL of air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
5. Load the cartridge with 380 µL of Cartridge Wash Buffer 2 using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** 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™ HT Xpress System. If you overestimate the cell count, you will underload cells in the BD Rhapsody™ 8-Lane 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 24.
- Do not rely on fluorescence-activated cell sorter-based (FACS-based) counts, because cell concentration might be overestimated by this method. Always recount cells after FACS using one of the recommended methods in Cell counting methods on page 24.
- 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 24.
- 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, use the appropriate settings for 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 26. 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 24
- Counting cells by Trypan Blue staining and brightfield microscopy on page 25
- Counting cells by automated cell counting on page 26

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**.

   Note: Performance might be impacted if samples are not in Sample Buffer. For rare samples that are not resuspended in Sample Buffer before cell loading, proceed at your own risk or contact tech support.

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.

   Notes:

   - For low abundance or low volume samples, filtering is optional at this step.
   - Count cells immediately.
   - Keep cells on ice, and protect them from light.

6. Completely resuspend cells gently using a 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 26.

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.

2. Completely resuspend cells gently using a 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 26.

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 24.

Preparing a single-cell suspension for cartridge loading

1. Determine the desired number of cells to capture in the BD Rhapsody™ 8-Lane 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,b]	Estimated multiplet rate
100	0.02%
500	0.1%
1000	0.2%
2000	0.4%
3000	0.6%
4000	0.7%
5000	0.9%
6000	1.1%
7000	1.3%
8000	1.5%
9000	1.7%
10000	1.9%
11000	2.0%
12000	2.2%
13000	2.4%
14000	2.6%
15000	2.8%
16000	3.0%
17000	3.2%
18000	3.3%
19000	3.5%
20000	3.7%
25000	4.6%
30000	5.5%
35000	6.4%

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

Number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads (target)[a,b]	Estimated multiplet rate
40000	7.3%
45000	8.2%
50000	9.1%
55000	10.0%

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 100–55,000 cells dependent on the library preparation.

b. This sample calculator gives loading calculations based on total cell count, which does not consider cell viability. The number of viable cells captured in the cartridge might be less than the targeted number of captured cells if the viability of the sample is <100%.

2. Determine the pooling ratio of samples to load onto the BD Rhapsody™ 8-Lane 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 for a single lane (µL)

   N = desired number of captured cells in cartridge

   P = pooling ratio

   C = total cell concentration (cells/µL)

Example

On a BD Rhapsody™ 8-Lane 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 / 200 cells/µL= 34 µL

Volume of sample B needed = 10,000 cells x 0.5 x 1.36 / 400 cells/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 380 µL. Using the example in **step 4**:

   B = 380 µL – 51 µL= 329 µL

   Note: For low abundance samples, if the ratio of cells and targeted number of cells cannot be achieved, mix cells to prepare the maximum input of cells in a total volume of 380 µL.

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. Proceed immediately to Processing cells with BD Rhapsody™ HT Xpress System on page 30.

6. Processing cells with BD Rhapsody™ HT Xpress System

Loading cells in BD Rhapsody™ 8-Lane Cartridge

Best practices

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

Before you begin

- Prime and treat the BD Rhapsody™ 8-Lane Cartridge. See Preparing BD Rhapsody™ 8-Lane 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 cartridge

1. Transfer each sample of cell suspension to a 96-deep well plate for multiple lane loading. Keep on ice.
2. Load the cartridge on the tray with 380 µL of air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
3. Change the mode of the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Load**.
4. With a manual pipette, gently pipet the cell suspension up and down to mix.
5. On the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX), press the pipette button once to aspirate 320 µL of the cell suspension.
6. Insert the tip(s) of the pipette perpendicular to the port, seal the pipette tip against the gasket, and then dispense 320 µL of the cells into the cartridge.

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

7. Leave the cartridge with loaded cells on the tray at room temperature (15–25 °C) for 8 minutes.
8. During the 8-minute incubation on the laboratory bench, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See Preparing BD Rhapsody™ Enhanced Cell Capture Beads on page 32.

Preparing BD Rhapsody™ Enhanced Cell Capture Beads

Before you begin

- Clean the pre-amplification workspace for preparation of the BD Rhapsody™ Enhanced Cell Capture Beads for the BD Rhapsody™ 8-Lane 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.

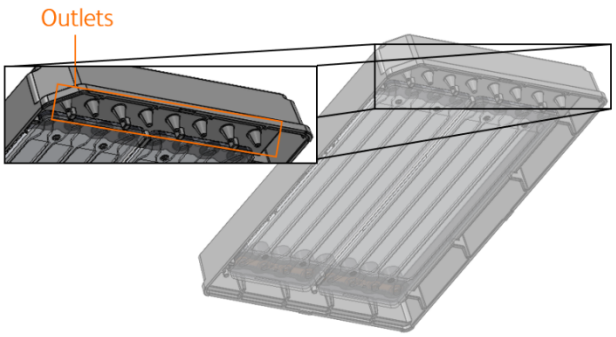
1. Place the tube with BD Rhapsody™ Enhanced Cell Capture Beads on the magnet for 1 minute.
2. Carefully remove and appropriately discard the storage buffer without disturbing the beads and while leaving the tube on the magnet.
3. Remove the tube from the magnet, and then pipet 380 µL of cold Sample Buffer into the tube of beads.
4. Pipet the bead suspension up and down to mix.
5. Keep the beads on ice.
6. Transfer each tube of the BD Rhapsody™ Enhanced Cell Capture Beads to a 96-deep well plate for multiple lane loading. Keep on ice.

Loading BD Rhapsody™ Enhanced Cell Capture Beads on to BD Rhapsody™ 8-Lane Cartridge

1. Change the mode of the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Prime/Wash**.
2. Load the cartridge with 380 µL of air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
3. Change the mode of the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Mix** mode.
4. Use the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Mix** mode to gently pipet the BD Rhapsody™ Enhanced Cell Capture Beads six times or until beads are completely resuspended in cold Sample Buffer. Make sure that the pipette tips are reaching the bottom of the wells to mitigate the chance of introducing air bubbles. Discard used pipette tips.
5. With new pipette tips, set the pipette to **Load** mode.
6. Immediately load. Check the pipette tips to make sure that there are no air bubbles inside the tips before loading. Otherwise, dispense in the 96-deep well plate and aspirate with a new set of pipette tips to reload.
7. Using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Load** mode, immediately load the cartridge with 320 µL of beads.
8. Let the beads settle in the cartridge on the tray at room temperature (15 °C to 25 °C) for 3 minutes.
9. Place the cartridge on the plate shaker, and secure it on the plate adapter.
10. Shake the cartridge at room temperature (15–25 °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.

11. 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 wipe.



12. Return the cartridge to the tray of the BD Rhapsody™ HT Xpress System.
13. Aliquot Sample Buffer in 10-mL reagent reservoir as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table:

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
Sample Buffer	0.76	2.00	2.80	3.60	4.30	5.10	5.90	6.60

14. Change the mode of the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Prime/Wash**.
15. Load the cartridge with 380 µL of air using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
16. Load the cartridge with 380 µL of cold Sample Buffer using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Prime/Wash** mode.
17. 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 4 or 8 lanes 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 15-mL Lysis Buffer bottle.

Note: Use the Lysis Buffer with DTT within 24 hours, and then discard.

2. Briefly vortex lysis mix, and aliquot in 10-mL or 25-mL reagent reservoir as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
Lysis Buffer	0.28 / 1.00	3.75	5.60	7.50	9.40	5.10	13.10	15.00

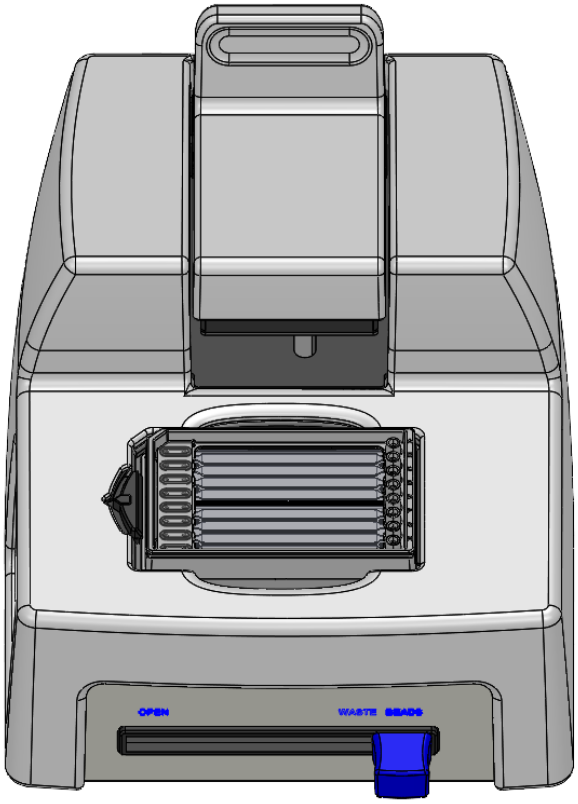
3. Return the cartridge to the tray of the BD Rhapsody™ HT Xpress System.
4. Set the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Lysis** mode.
5. Load the cartridge with 280 µL of Lysis Buffer with DTT using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Lysis** mode.
6. Incubate at room temperature (15–25 °C) for 2 minutes.

   Maintain the recommended lysis time for best performance.

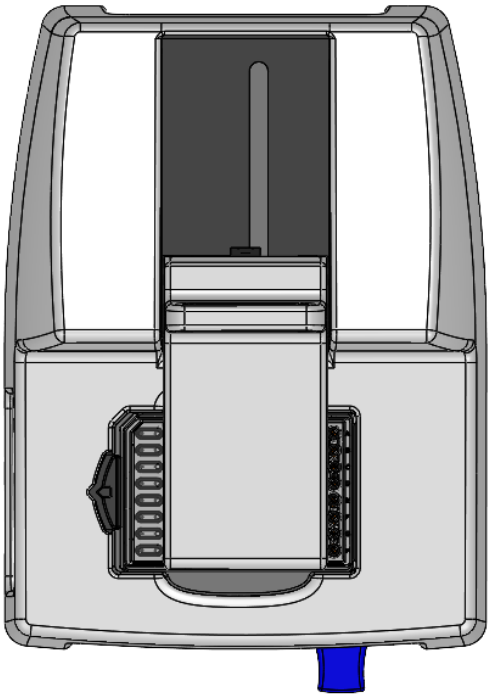
   Note: Before retrieval, remove extra buffer that has pooled at the inlet with a P20 pipette to minimize overflow. Aspirate at an angle to avoid accidental aspiration of buffer volume in the microwell array.

Retrieving BD Rhapsody™ Enhanced Cell Capture Beads from cartridge

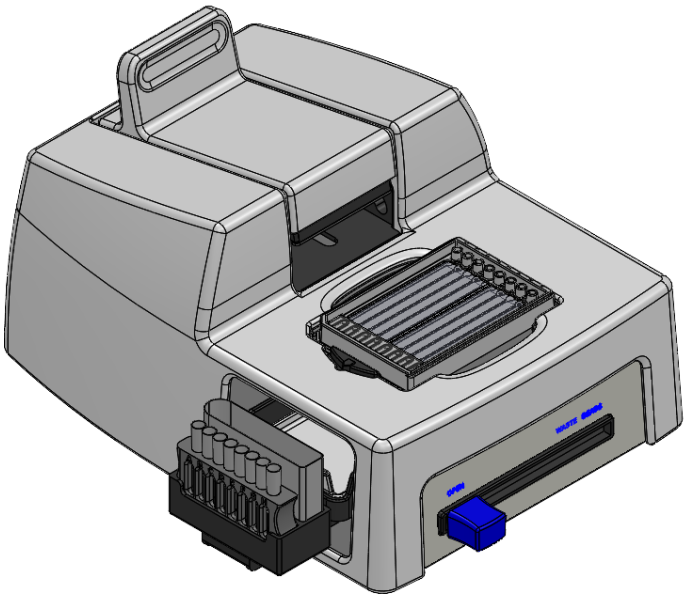
1. Confirm that a cluster tube was placed in the BD Rhapsody™ HT Xpress System drawer for bead retrieval. Label the tubes appropriately.
2. Move the front slider to **BEADS** on the BD Rhapsody™ HT Xpress System.



3. Gently pull the top **RETRIEVAL** slider onto the BD Rhapsody™ 8-Lane Cartridge (ACTIVE). Make sure that the Retrieval magnet is in contact with the BD Rhapsody™ 8-Lane Cartridge.



4. Leave Retrieval magnet in **ACTIVE** position for 1 minute.
5. Set the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Retrieval** mode.
6. Aspirate 1000 µL Lysis Buffer with DTT with the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) in **Retrieval** mode.
7. Press down on the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to seal against the gasket.
8. Push back the top RETRIEVAL magnet (INACTIVE) and immediately load 1000 µL Lysis Buffer with DTT.
9. Remove the pipette from the gasket and purge the tip.
10. Move the front slider to **OPEN**, remove the cluster tube with the bottom adapter to a flat, secure surface.



11. Move the front slider to **WASTE**. Do not throw away the Waste container.
12. Blot the outlet drip on the bottom of the cartridge with a lint-free wipe to remove residual liquid.
13. Immediately proceed to Washing BD Rhapsody™ Enhanced Cell Capture Beads on page 37.
14. Keep partially used cartridge on a flat surface while Washing BD Rhapsody™ Enhanced Cell Capture Beads on page 37.
15. Perform "Washing used lanes and BD Rhapsody™ 8-Lane Cartridge storage procedure" during cDNA synthesis. See the *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol*.
16. Appropriately dispose of the BD Rhapsody™ 8-Lane Cartridge according to biosafety level (BSL) when all 8 lanes have been used.

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 53.

17. Appropriately dispose of the Lysis Buffer with DTT.
18. Wipe the BD Rhapsody™ HT Xpress System with 10% (v/v) bleach or 70% (v/v) ethyl alcohol. See the *BD Rhapsody™ HT Xpress System Installation and Maintenance Guide*.
19. Clean the partially used cartridge following the procedures in "Washing used lanes and BD Rhapsody™ 8-Lane Cartridge storage procedure." See the *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol*.

Washing BD Rhapsody™ Enhanced Cell Capture Beads

Notes:

- Keep the BD Rhapsody™ Enhanced Cell Capture Beads cold during washes.
- Use low-retention tips to handle BD Rhapsody™ Enhanced Cell Capture Beads.

1. Gently pipet-mix the retrieved BD Rhapsody™ Enhanced Cell Capture Beads from the cluster tube and transfer them separately into a new 1.5-mL LoBind tube.
2. If there are still beads left in the cluster tube, add 100 µL of Lysis Buffer with DTT, rinse the cluster tubes, and transfer to the same 1.5-mL LoBind tube from the previous step. Keep tubes on ice.
3. Place the tube on the 1.5-mL tube magnet for 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads, while leaving the tube on the magnet.

   Note: Avoid leaving Lysis Buffer or bubbles in the tube. Lysis Buffer may cause the reverse transcription reaction to fail.
4. Remove the 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.
5. Place the tube on the 1.5-mL tube magnet for 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads, while leaving the tube on the magnet.
6. Remove the 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.

Proceed to cDNA synthesis. See the *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol*.

If profiling TCR/BCR, see the appropriate BD Rhapsody™ TCR/BCR Full Length Library Preparation Protocol.

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 inspection by microscopy first before proceeding to cartridge preparation troubleshooting.

Inspection by microscopy: Introduction

Inspection 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.

Inspection by microscopy requires that you stain cells with viability stains, Calcein AM and Propidium Iodide, before loading the cell sample in the BD Rhapsody™ 8-Lane 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).

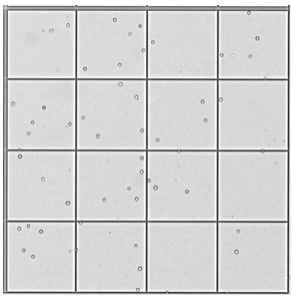
Check the BD Rhapsody™ 8-Lane Cartridge under a microscope after cell loading, bead loading, second bead wash, and retrieval steps. If possible, take brightfield and fluorescence microscopic images at each step.

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 49.

Inspection 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.

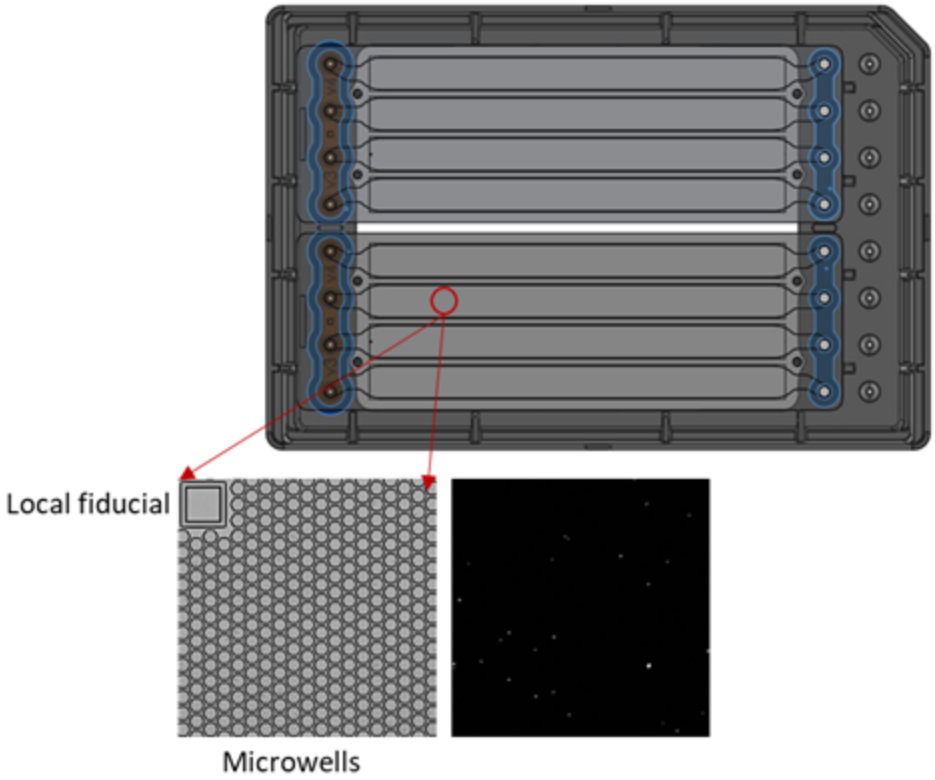


Inspection by microscopy: Cartridge inspection after Cell Load

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 8 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, second Bead Wash, and Bead Retrieval steps in the BD Rhapsody™ workflow.
3. Image the BD Rhapsody™ 8-Lane 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 40] 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 41 through Region 5: 26 cells on page 42]:

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**

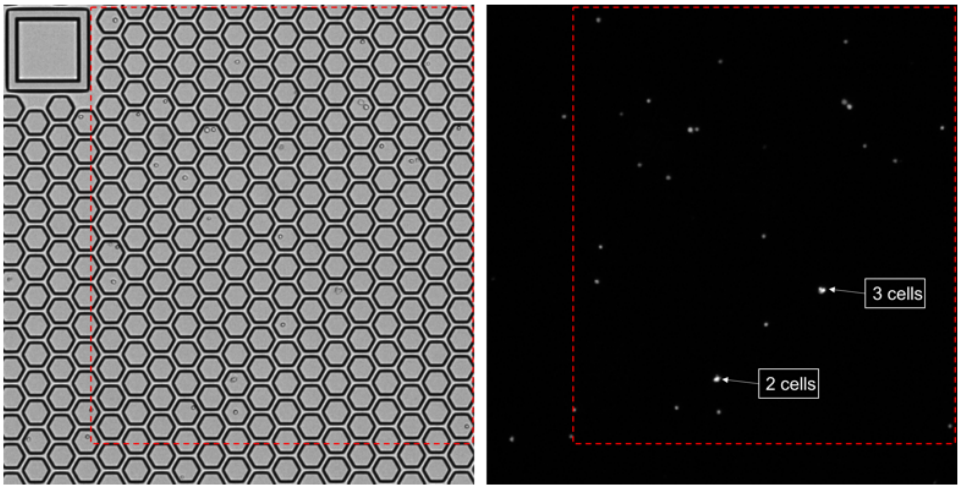
Therefore, accounting for five regions:

Cells/well = 105/1,125 = 0.0933

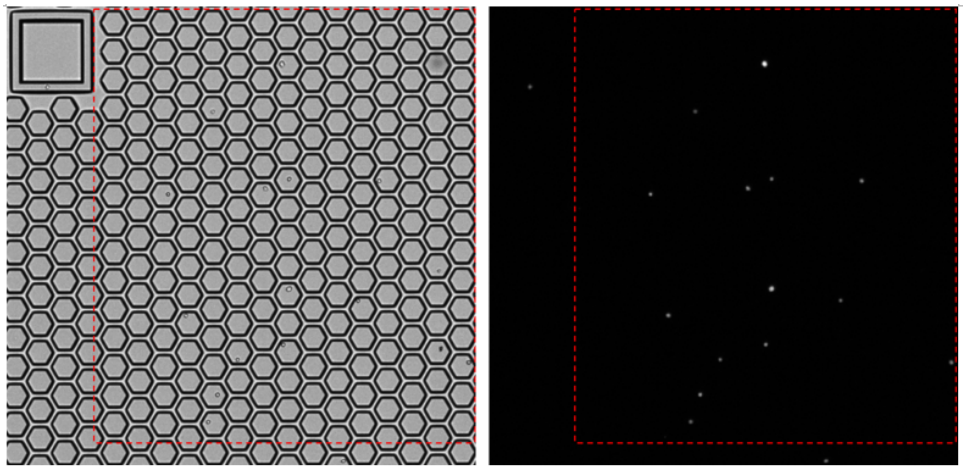
Total no. cells captured in the cartridge = 0.0933 x 221,891

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

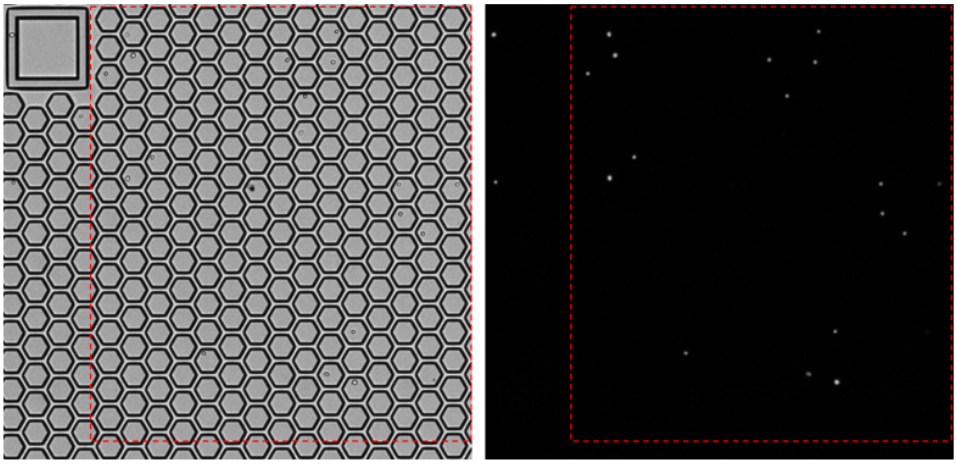
Region 1: 26 cells



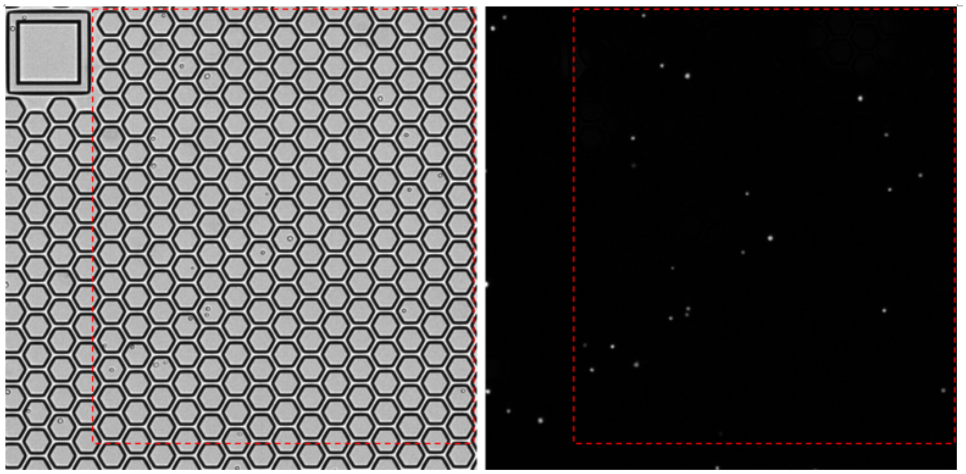
Region 2: 14 cells



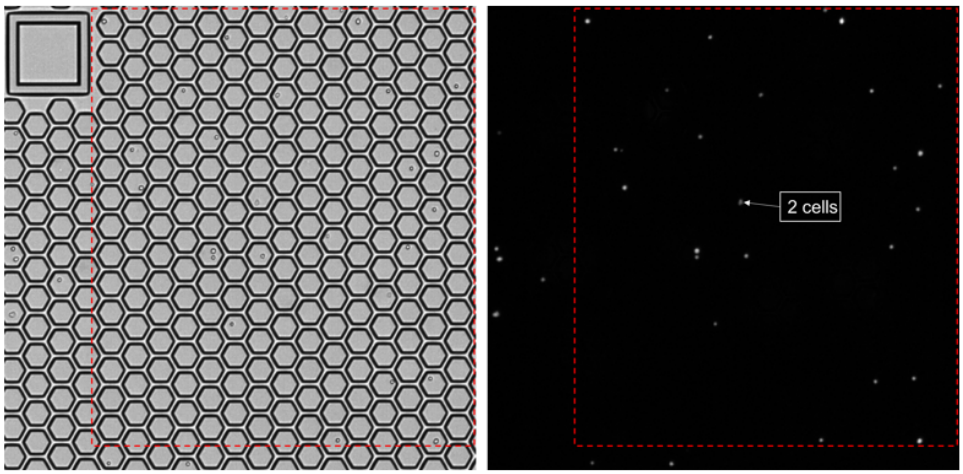
Region 3: 17 cells



Region 4: 22 cells



Region 5: 26 cells



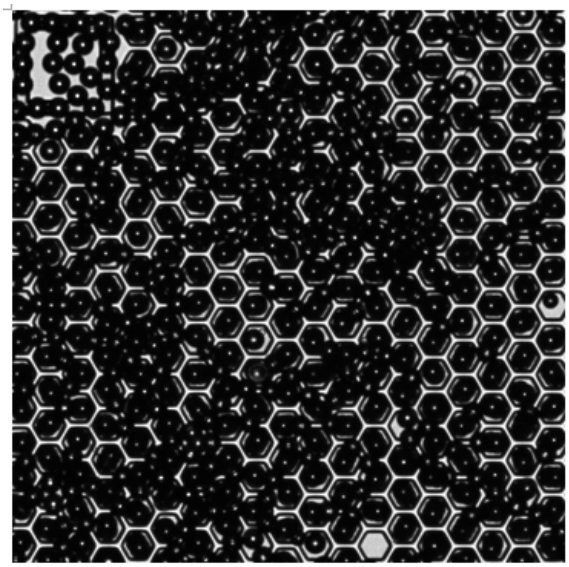
Inspection by microscopy: Cartridge inspection after Bead Load

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™ 8-Lane 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 absence of beads in brightfield images might indicate insufficient bead loading.

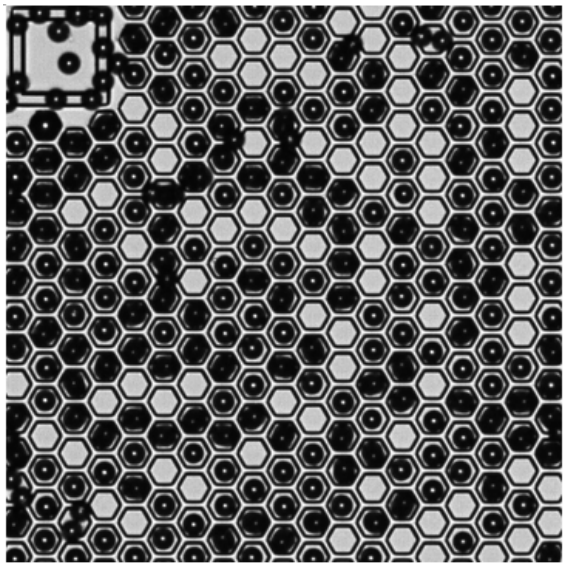
Example images after Bead Load

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



Inspection 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™ 8-Lane 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

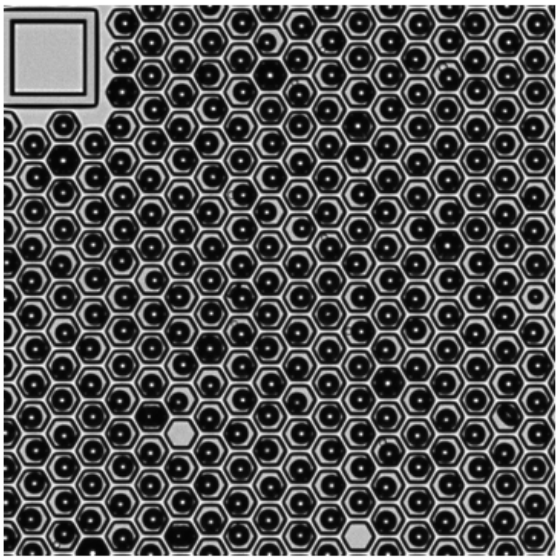
4. Estimate the total number of cells captured in the cartridge. See Estimate cell viability and the total number of cells captured on page 39. You will use the total number of cells captured in the cartridge in **step 6**.
5. Use the concentration of cell suspension at Cell Load to find the estimated multiplet rate. See the multiplet rate table in Preparing a single-cell suspension for cartridge loading on page 27. 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)

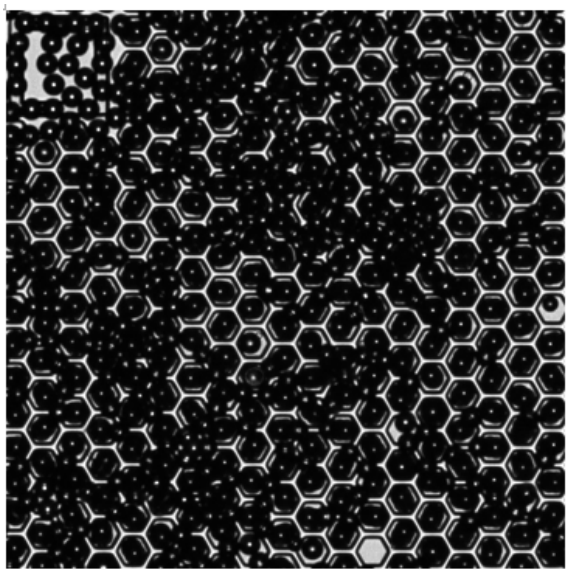
Example images after Bead Wash

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

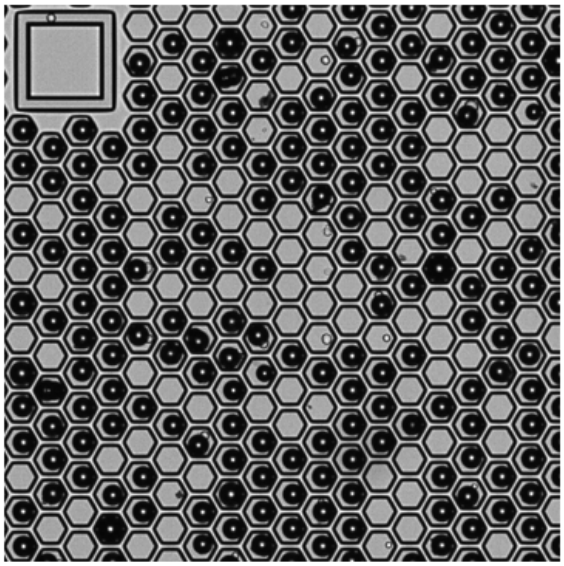
Acceptable bead number after the second bead wash



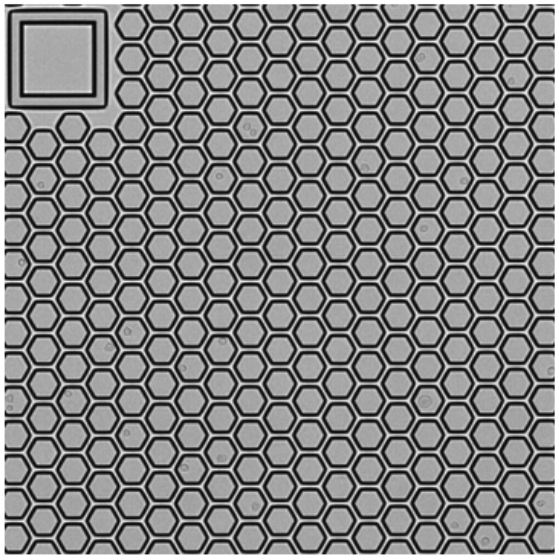
Insufficient removal of excess BD Rhapsody™ Enhanced Cell Capture Beads



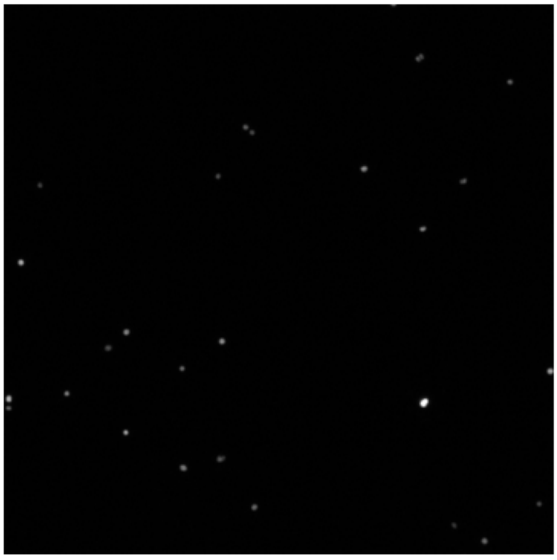
Significant loss of BD Rhapsody™ Enhanced Cell Capture Beads resulting in empty microwells after the second bead wash



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

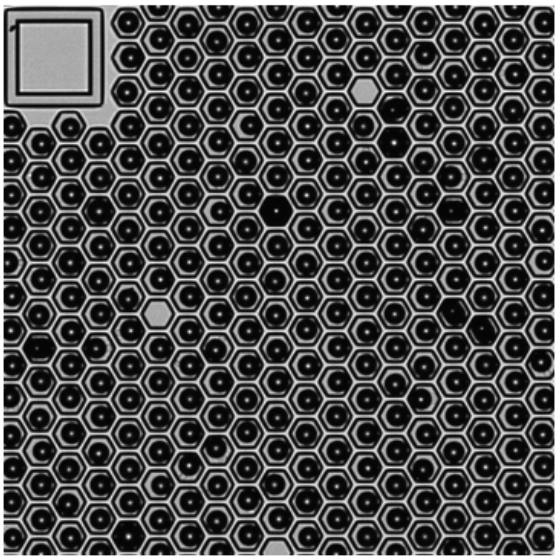


Brightfield



Fluorescent

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



Brightfield

Fluorescent

Inspection 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

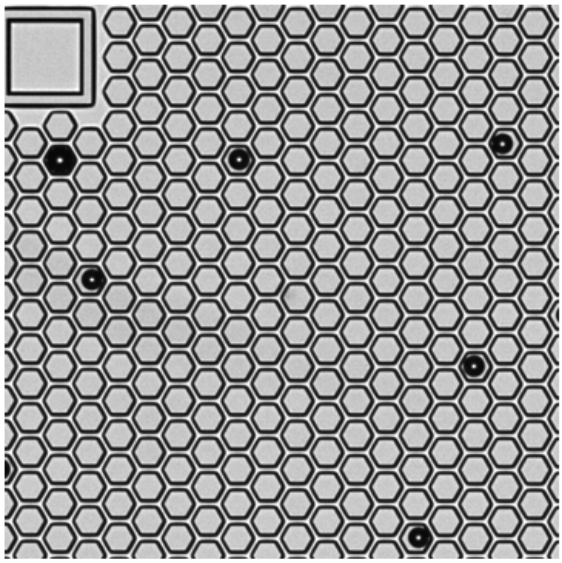
1. After bead retrieval is complete, image the BD Rhapsody™ 8-Lane Cartridge under a brightfield microscope. Quantities of beads observed in brightfield images might indicate insufficient bead retrieval.
2. Calculate the remaining beads in the cartridge after bead retrieval:
   a. Count the number of beads in ≥200 wells.
   b. Calculate:

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

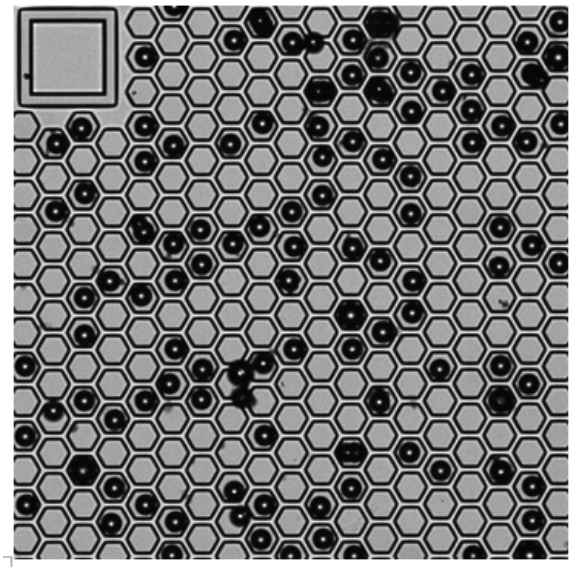
Example images after Bead Retrieval

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



Inspection by microscopy: BD Rhapsody™ 8-Lane 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™ 8-Lane 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 introduction of air bubbles from entering the cartridge.

Cartridge preparation troubleshooting

First, perform inspection by microscopy to identify issues with the cartridge workflow before using the cartridge preparation troubleshooting tables in this section. See Inspection by microscopy: Introduction on page 38. 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 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™ 8-Lane 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 23.
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 23.
	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 42. • 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 **Load** mode on the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX).

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 44. • Repeat the two bead wash steps after shaking the cartridge on the plate shaker.
	Wrong pipette mode.	• Use the Prime/Wash mode on the BD Rhapsody™ P8xP1200µL Pipette – HTX for the two bead wash step.
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 44 and Significant loss of BD Rhapsody™ Enhanced Cell Capture Beads resulting in empty microwells after the second bead wash on page 45. • 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 Prime/Wash mode on the BD Rhapsody™ P8xP1200µL Pipette – HTX for the two bead wash steps.
	Wrong pipette mode.	• Repeat the experiment with a new cartridge. Use the Prime/Wash mode on the BD Rhapsody™ P8xP1200µL Pipette – HTX 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 51 and Significant cell loss after the second bead wash: Images after the second bead wash on page 46. • 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 8 minutes and not less.

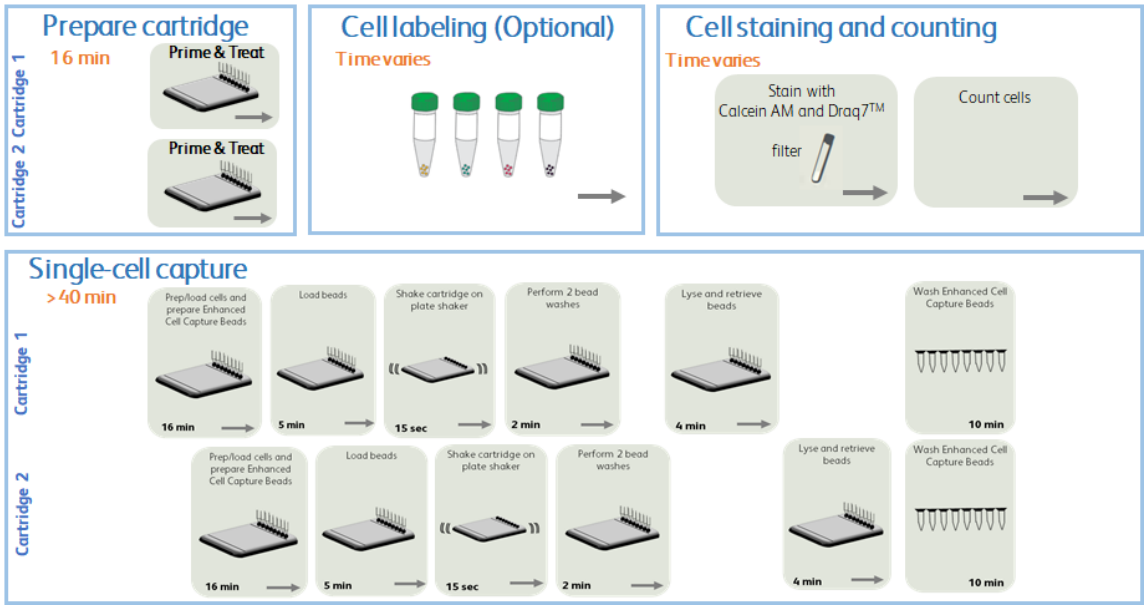
Cartridge preparation troubleshooting

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 47. • 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™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX).
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™ 8-Lane Cartridges

Workflow with two cartridges

Staggered boxes indicate staggering the start of the same steps.



9. Safety

General safety and limitations

For instrument safety, see the *BD Rhapsody™ HT Xpress System 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™ HT Xpress 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™ HT Xpress System 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™ HT Xpress System. For more information, see Instrument technical support on page 6.

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