 **BD** Rhapsody™ HT Single-Cell  
Analysis System  
Instrument User Guide

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## Regulatory information

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

## History

Revision	Date	Change made
23-24257(01)	2023-02	Initial release.

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# 1. Introduction

## About this guide

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This guide provides instructions for operating the BD Rhapsody™ HT Single-Cell Analysis System, as well as supporting materials.

To use the BD Rhapsody™ HT Xpress System without the BD Rhapsody™ Scanner see the *BD Rhapsody™ HT Xpress System Instrument User Guide*.

Single-Cell Multiomics technical publications are available for download from the BD® Single-Cell Multiomics Resource Library at [scmix.bd.com/hc/en-us/categories/360000838932-Resource-Library](https://scmix.bd.com/hc/en-us/categories/360000838932-Resource-Library).

## Intended use

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The BD Rhapsody™ HT Single-Cell Analysis System is intended for the preparation of single-cell sequencing libraries. The BD Rhapsody™ Scanner uses brightfield and dual band fluorescence to count cells and measure cell viability. Proprietary BD® Molecular Indexing technology is used to count individual mRNA molecules. Cells are entirely contained in each of the 8 lanes of the BD Rhapsody™ 8-Lane Cartridge, where each lane 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™ HT Single-Cell Analysis System.

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

For more information on the purpose of the instruments, see [BD Rhapsody™ HT Xpress System overview on page 9](#) and [BD Rhapsody™ Scanner overview on page 10](#).

## Restrictions

Any use of the BD Rhapsody™ HT Single-Cell Analysis 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 Single-Cell Analysis System.
- Unauthorized alterations or adjustments to instrument hardware or software.
- 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 Single-Cell Analysis System warranty.

## Disclaimer


The instrument, external components, software, and consumables in the BD Rhapsody™ HT Single-Cell Analysis 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

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For safety and limitations, see the *BD Rhapsody™ HT Single-Cell Analysis System Safety and Limitations Guide*.

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

Symbol	Meaning
	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

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Before handling chemicals, read and understand the Safety Data Sheets (SDSs). To obtain SDSs for chemicals ordered from BD, go to [regdocs.bd.com](http://regdocs.bd.com), or contact your local Field Application Specialist (FAS) or [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).

## Instrument technical support

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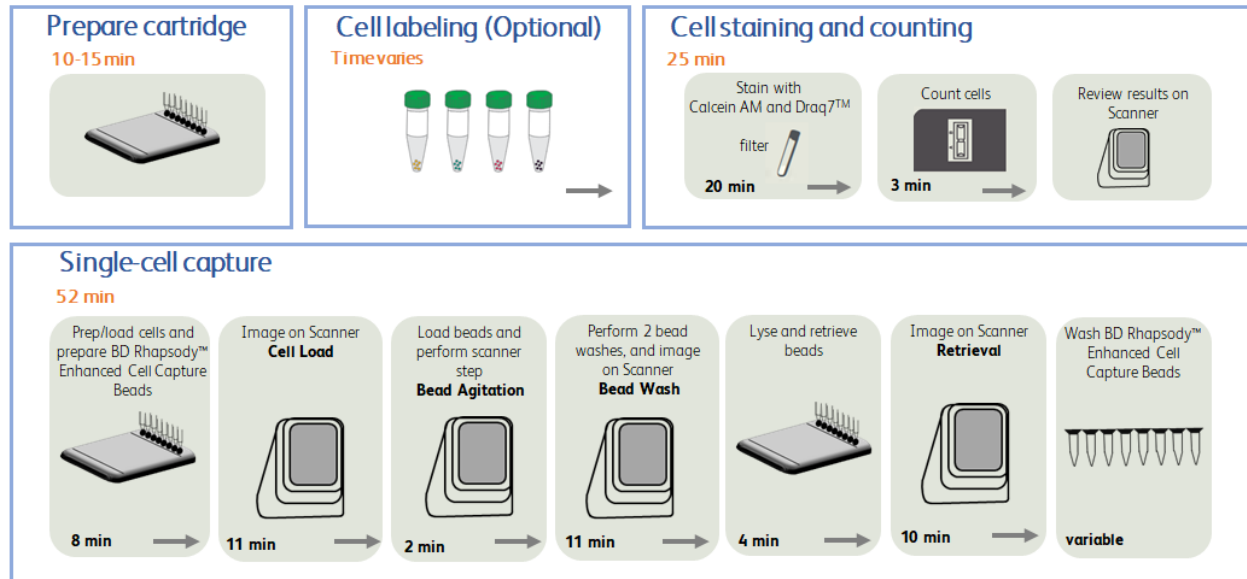
If technical assistance is required, contact your local Field Application Specialist (FAS) or [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) or 1.877.232.8995. You can contact technical support in Europe at [help.biosciences@bd.com](mailto:help.biosciences@bd.com) or +32.53.720.600.

Before contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number or lot number
- Any error messages
- Details of recent system performance
- For the BD Rhapsody™ Scanner, the version of the software that you are using

## 2. Getting Started

### BD Rhapsody™ 8-Lane Cartridge workflow



### 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 Single-Cell Analysis System Site Preparation Guide* and [BD Rhapsody™ Scanner metrics on page 57](#).

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 [scmix.bd.com/hc/en-us/categories/360000838932-Resource-Library](https://scmix.bd.com/hc/en-us/categories/360000838932-Resource-Library).

## Pipette settings

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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

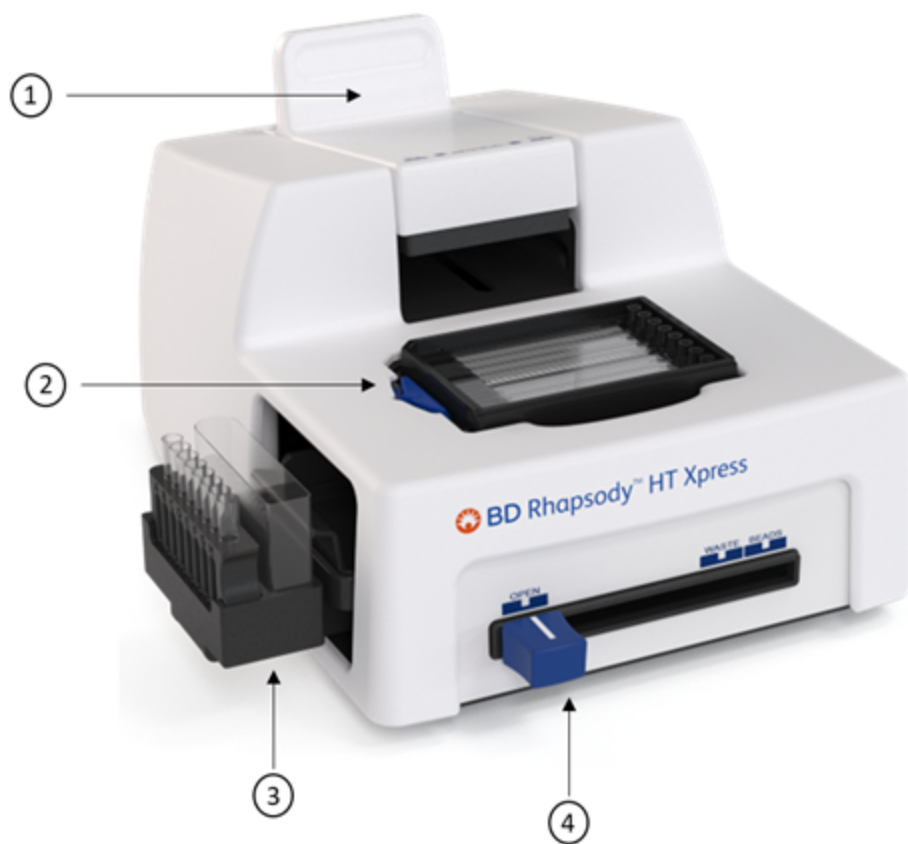


## BD Rhapsody™ HT Xpress System overview

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 Single-Cell Analysis 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).

## BD Rhapsody™ Scanner overview

The BD Rhapsody™ Scanner is used to count cells by brightfield and dual band fluorescence imaging and calculate volumes of cells and Sample Buffer needed to prepare single-cell suspensions.

For safety and limitations of the scanner, see the *BD Rhapsody™ HT Single-Cell Analysis Safety and Limitations Guide*.

See the *BD Rhapsody™ HT Xpress Installation and Maintenance Guide* to review critical installation and periodic maintenance procedures on the scanner.

The following figure shows the main components of the scanner for operation:



No.	Front component
1	Scanner touchscreen.
2	Scanner front power button and standby switch. Lit green: ON. Flashing: PC asleep.
3	USB 3.0 port.
4	Cartridge loading door where a tray extends and retracts.

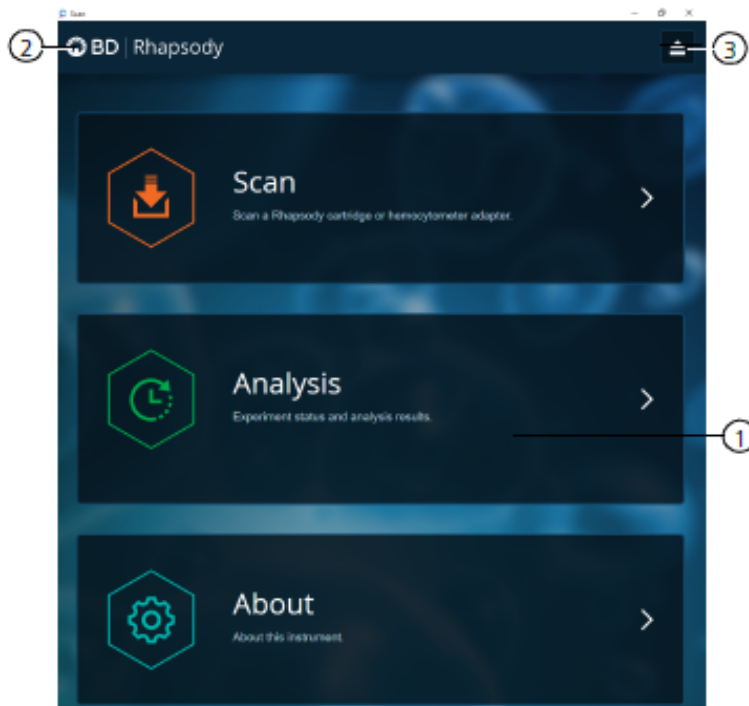


No.	Rear component
1	Master power switch
2	Fuse holder
3	24 VDC power input
4	USB 2.0 ports (2) <sup>a</sup>
5	Ethernet port

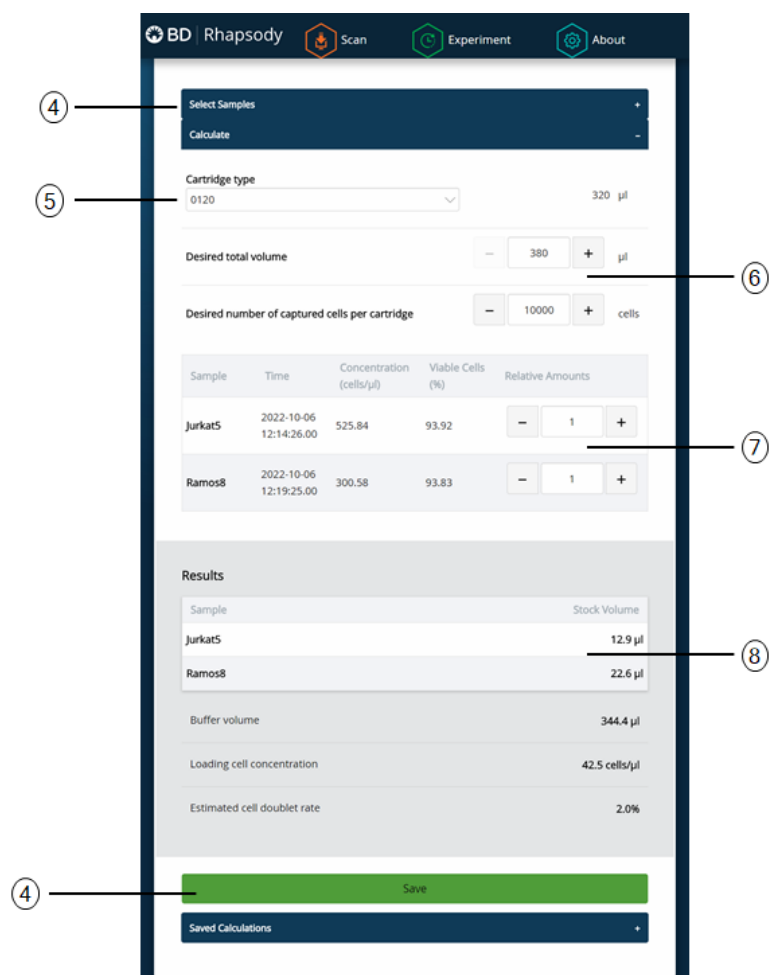
a. The USB 2.0 ports on the rear of the instrument are primarily used by BD Biosciences technical support.

# Quick guide to BD Rhapsody™ Scanner software

## Navigation



No.	Description
1	Tap an application (app) to navigate between scan, analysis, and about screens.
2	Tap <b>BD Rhapsody</b> on any screen to return to the main screen.
3	(Optional) Tap the eject icon to eject the tray from the sample loading door of the scanner. The scanner automatically ejects the tray.



No.	Description
4	Tap <b>Select Samples</b> to display the window for selecting samples. Tap <b>Calculate</b> to calculate volumes for preparing cell suspensions that have been scanned using the hemocytometer protocol. Tap <b>Save</b> on the Calculate tab to display saved calculations.
5	From the drop down, select the <b>cartridge type</b> depending on whether a single-lane or 8-lane cartridge is used. 0109 is used for a single-lane cartridge and 0120 is used for an 8-lane cartridge.
6	Enter the total volume and desired number of captured cells to prepare the cell suspension for loading into the BD Rhapsody™ Cartridge.
7	(Optional) Enter the relative amounts of samples that comprise the cell suspension. The default ratio is 1:1.
8	Obtain the volumes of buffer and stock cells required to prepare the cell suspension for cartridge loading.

## Best practices

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### 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.

### 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 20](#).
- 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.

## BD Rhapsody™ Scanner software

For easy access to analysis metrics, place the Rhapsody Data folder into the Quick Access folder:

1. Open a file explorer window.
2. Enter Public Documents in the location box.
3. Drag the Rhapsody Data folder to Quick Access.
4. Open the Rhapsody Data folder.
5. Open the folder for the appropriate experiment.
6. Open the .csv files with Microsoft® Excel.

## 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 <sup>a</sup>	Thermo Fisher Scientific	C1430
DRAQ7™ <sup>a</sup>	BD Pharmingen™	564904
Dimethyl sulfoxide (DMSO)	Major supplier	–
70% ethyl alcohol or 70% isopropyl alcohol <sup>b</sup>	–	–

a. Protect Calcein AM and DRAQ7™ 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)	Thermo Fisher Scientific	F171803G
Or, ZAP™ SLIK 1000 µL low retention aerosol filter pipette tips for BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) (alternative)	Labcon	1177-965-008-9
60 mL reagent reservoir self-standing <sup>a</sup>	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 <sup>b</sup>	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	–
<p>a. Waste collection container for the BD Rhapsody™ HT Xpress System.  b. Bead retrieval tubes for the BD Rhapsody™ HT Xpress System.</p>		

## 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™ Scanner	BD Biosciences	633701
BD Rhapsody™ HT Xpress <sup>a</sup>	BD Biosciences	666730
Hemocytometer adapter <sup>a</sup>	BD Biosciences	633703
BD Rhapsody™ P1200µL Pipette – HTX <sup>a</sup>	BD Biosciences	500066148
BD Rhapsody™ P8xP1200µL Pipette – HTX <sup>a</sup>	BD Biosciences	666718
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 <sup>b</sup>	Eppendorf	5360000038
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	New England Biolabs	S1506S
Or,		
12-Tube magnetic separation rack	New England Biolabs	S1509S
Or,		
Invitrogen™ DynaMag™-2 magnet	Thermo Fisher Scientific	12321D
<p>a. Part of the BD Rhapsody™ HT Xpress System. Items can be ordered separately.  b. For cDNA synthesis after cartridge cell capture.</p>		

## Suggested equipment

### Suggested equipment

Equipment	Supplier	Catalog no.
Logitech® wireless combo (keyboard and mouse).	Major supplier	MK270
Phase-contrast microscope.	Major supplier	–

## 4. Preparing BD Rhapsody™ 8-Lane Cartridge

### Before you begin

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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.

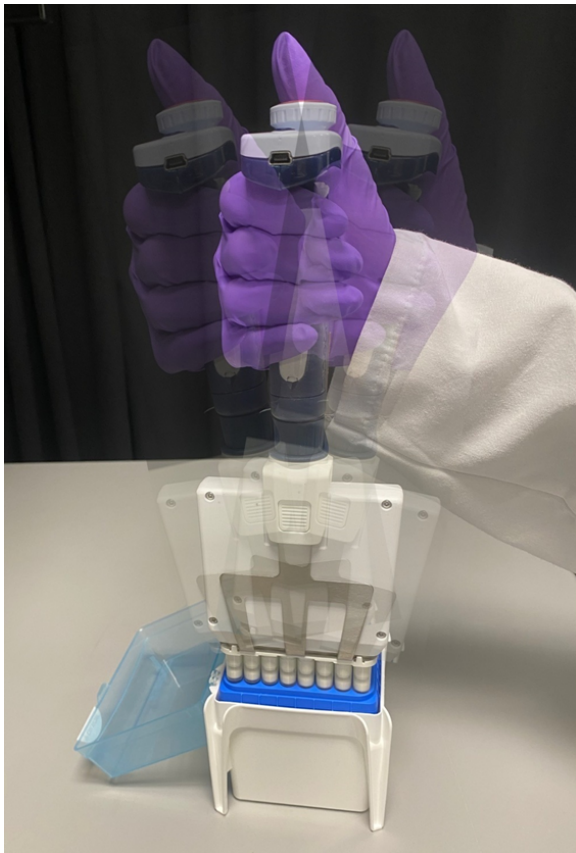
If cell preparation takes 4 hours or longer, begin preparing cells before cartridge preparation.

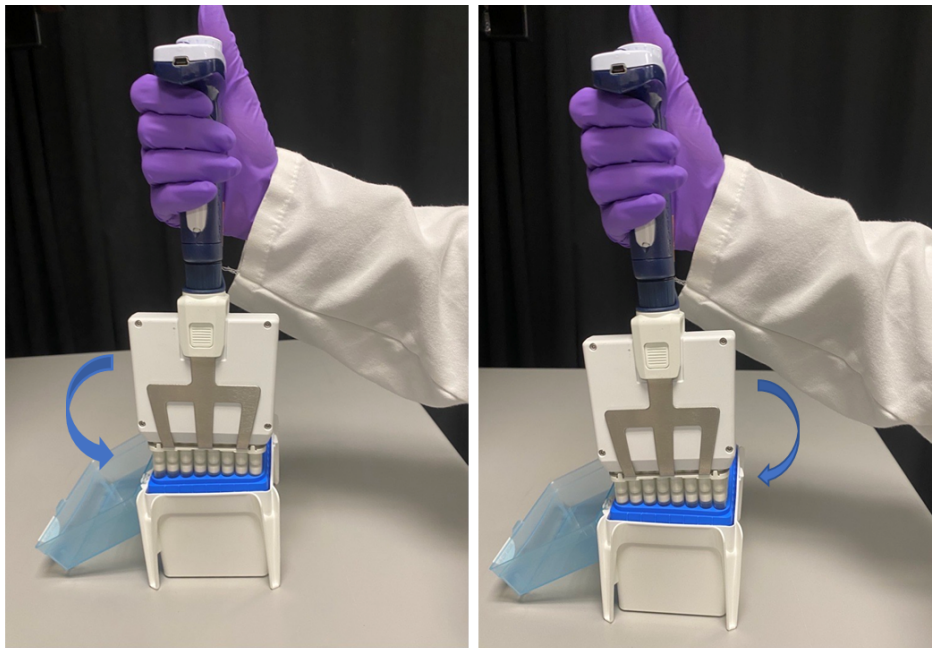
- Equilibrate these reagents at room temperature (15–25 °C) within 30 minutes before use:
  - Cartridge Wash Buffer 1
  - Cartridge Wash Buffer 2
- Place these reagents on ice:
  - Sample Buffer
  - BD Rhapsody™ Enhanced Cell Capture Beads
  - 1 M DTT
  - Lysis Buffer
- Thaw Calcein AM. Once at room 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 Single-Cell Analysis 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](#).

## Pipetting instructions

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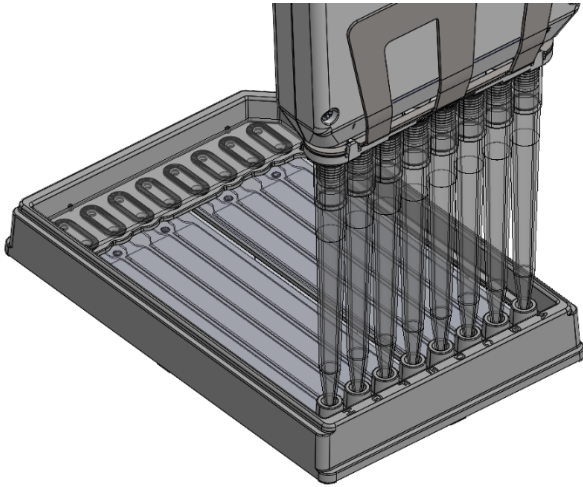
- For the BD Rhapsody™ P1200µL Pipette – HTX, push the tip holder into the tip using a slight twisting motion to ensure a firm and airtight seal.
- The battery is charged in the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX). Charging may only be carried out using the charging 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.

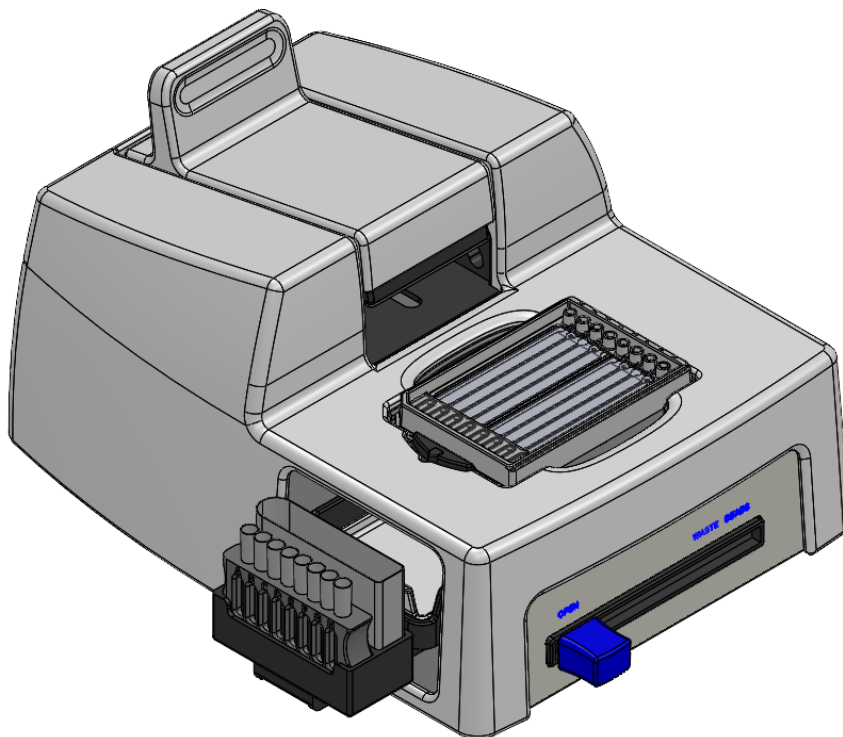


- Avoid introducing bubbles while pipetting into the BD Rhapsody™ 8-Lane Cartridge.
- Change pipette tips before every pipetting step.
- Always use low-retention filtered pipette tips and LoBind tubes.

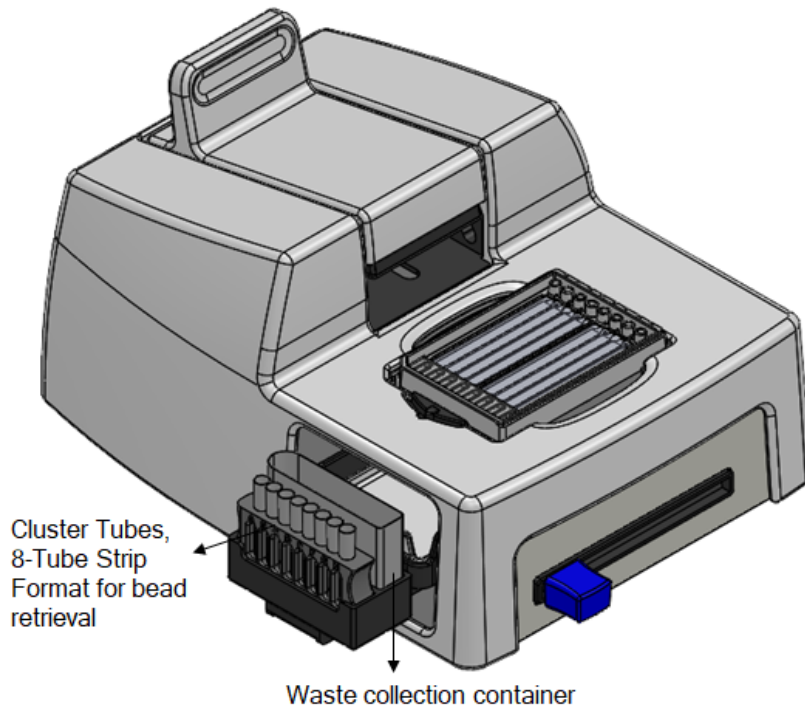
## Setting-up BD Rhapsody™ HT Xpress System

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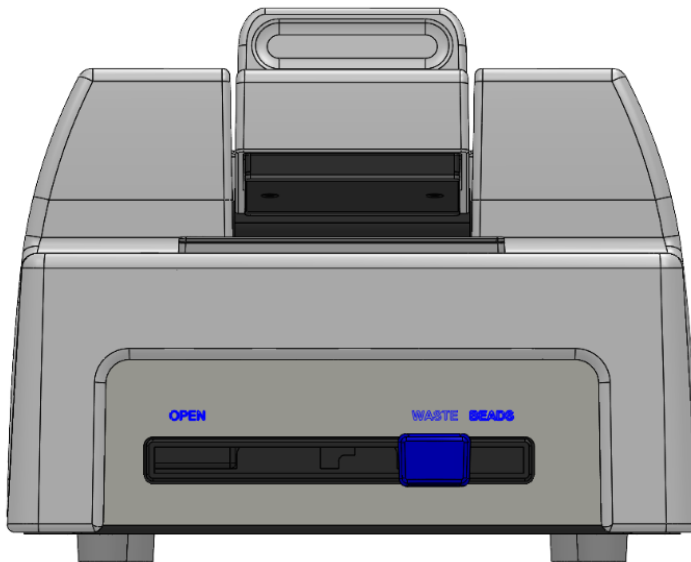
1. Make sure the Retrieval (top) magnet is away from the cartridge tray.
2. Move the front slider to **OPEN**:



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.

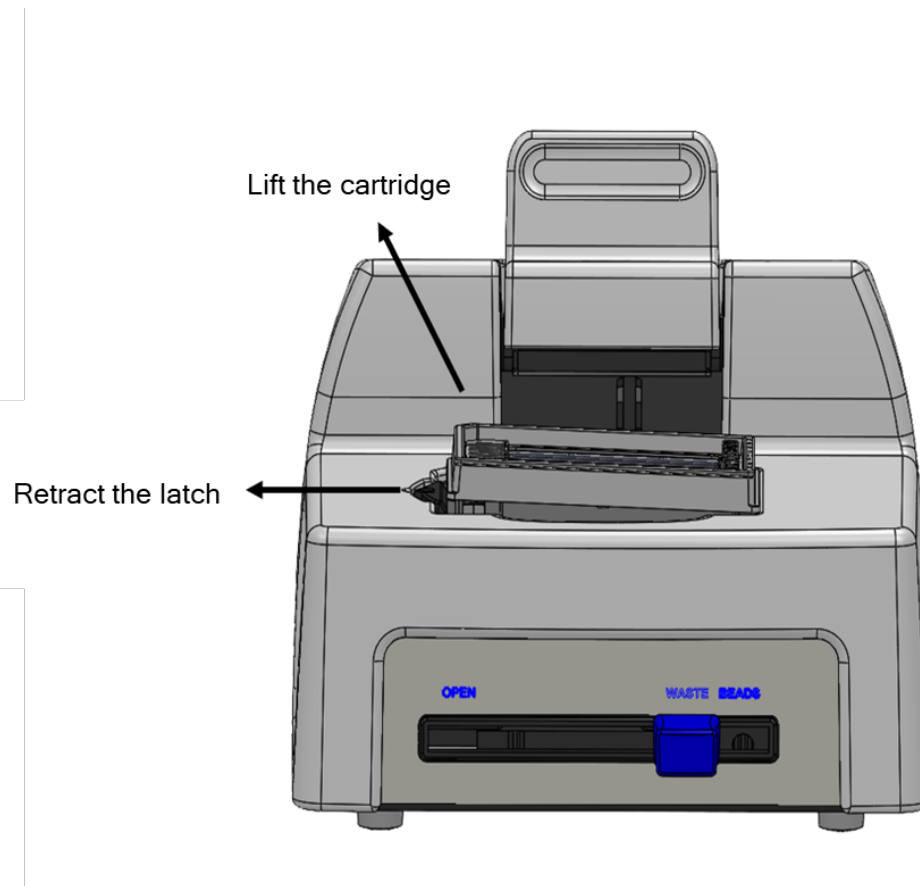


4. Move the front slider to **WASTE**:



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. Processing cells with BD Rhapsody™ HT Single-Cell Analysis System

## Best practices

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### 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, inspect the position of the cell pellet in the tube after centrifugation.

### Cell counting

- 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 (less than 2  $\mu\text{L}$ ).
- Cell counting the BD Rhapsody™ Scanner is most accurate when the cell concentration is in the range of ~200–800 cells/ $\mu\text{L}$ . If the cell concentration is greater than 1,000 cells/ $\mu\text{L}$ , dilute the cell suspension in cold Sample Buffer to ~200–800 cells/ $\mu\text{L}$ .
- 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.

## Staining cells with viability markers

---

### Before you begin

- Prepare a single-cell suspension.  
**Note:** If you are using biological samples that contain red blood cell contamination, red blood cell lysis is required. See the *Preparing Single-Cell Suspensions Protocol*.
- Protect Calcein AM and DRAQ7™ from light until ready to use.

### Procedure

1. If cells are not resuspended in cold Sample Buffer, centrifuge cell suspension at  $400 \times g$  for 5 minutes, aspirate supernatant, and leave ~20  $\mu\text{L}$  of residual supernatant. Add up to 620  $\mu\text{L}$  total volume of cold Sample Buffer, and then proceed to **step 2**. If the total expected cell number is less than or equal to 30,000 cells in 610  $\mu\text{L}$ , proceed to **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  $\mu\text{L}$  of 2 mM Calcein AM and 3.1  $\mu\text{L}$  of 0.3 mM DRAQ7™ to 620  $\mu\text{L}$  cell suspension (1:200 dilution) in cold Sample Buffer.
3. Gently pipet-mix.
4. Incubate at 37 °C in dark for 5 minutes.
5. Filter cells through Falcon® tube with Cell Strainer Cap.

**Note:** For low abundance or low volume samples, filtering is optional at this step.

6. Proceed immediately to [Loading cells into Hemocytometer Adapter on page 27](#).

## **Loading cells into Hemocytometer Adapter**

---

### **Before you begin**

Stain cells with viability markers. See [Staining cells with viability markers on page 26](#).

**Note:** Count cells immediately.

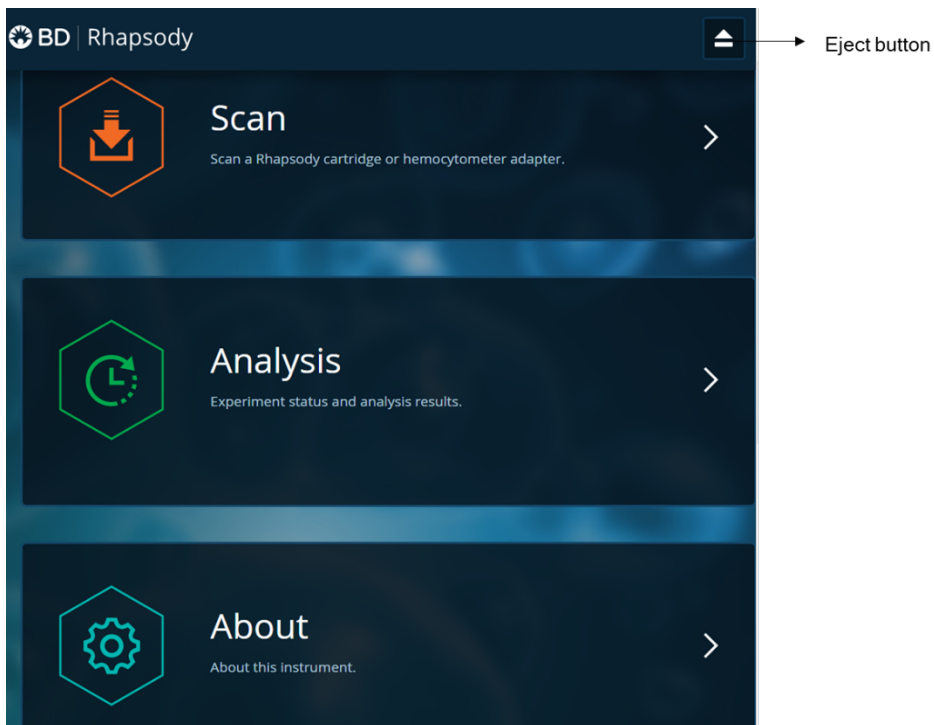
### **Loading cells**

**Note:** Keep cells on ice, and protect them from light.

1. Completely resuspend cells gently using a pipette, and then gently pipet 10  $\mu\text{L}$  of the cell suspension into one chamber of the INCYTO™ disposable hemocytometer.
2. Insert the hemocytometer into the Hemocytometer Adapter so that the A and B sides of the hemocytometer align with A and B on the Hemocytometer Adapter.
3. Count the cells in the BD Rhapsody™ Scanner.

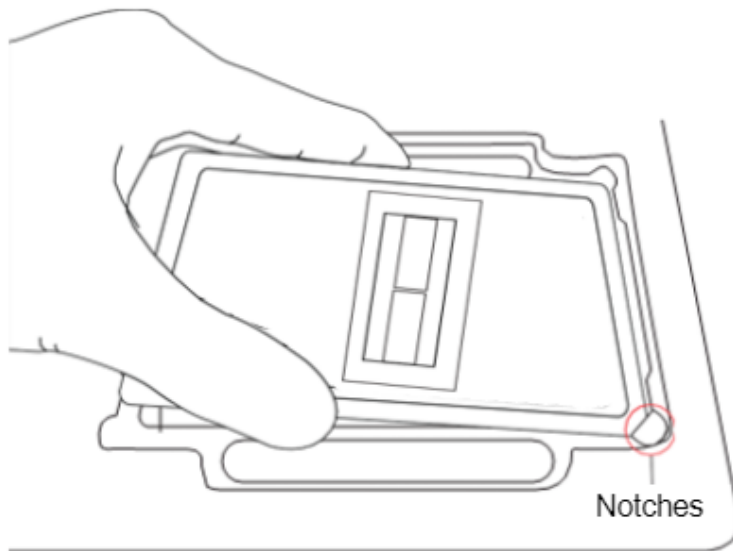
# Counting cells with BD Rhapsody™ Scanner

1. Launch the scanner software so that the main menu displays.



## Notes:

- To return to the main menu on any screen, tap the BD logo in the upper left.
  - For updates to the BD Rhapsody™ Scanner software, contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).
  - To troubleshoot scanner software error messages, see [BD Rhapsody™ Scanner software messages on page 54](#).
2. Tap **Scan**. The tray door of the scanner opens automatically, and the tray is ejected.
  3. Place the Hemocytometer Adapter on the scanner tray so that the notch of the adapter matches the corner notch, and the barcode faces toward the front of the instrument.

**Notes:**

- Make sure to place the adapter in the right orientation to avoid scanner not being able to detect the Hemocytometer.
  - You can manually enter the barcode in the application if necessary.
4. Tap **Continue**. The tray retracts, the door closes, and the scanner displays the hemocytometer setup screen.
  5. Select the protocol name as Hemocytometer (when using hemocytometer in the scanner) from the drop-down menu. Enter the experiment name, sample name and user. (If an experiment name was entered previously, select it from the drop-down menu.)

**Note:** Enter an experiment name appropriate for the hemocytometer and cartridge scans. If necessary, tap the touch keyboard icon in the bottom right on the screen to enter information. The length is limited to 32 characters and the name cannot start and end with spaces. In addition, the following special characters are prohibited < > [ ] / \ " \* ? | % , ; . ` ' .

Experiment: 20180131\_SL\_XH\_Stage-Shaking-Run3

Sample: Jurkat

Protocol: Hemocytometer

User: SL

Cartridge: 0107001033A

**Side A**

Start Side A Scan

**Side B**

6. Tap **Side A** or **Side B**, depending on which side the cells were loaded, and then Select **Start Side A Scan** or **Start Side B Scan**, as required.

**Note:** To stop the scan, tap **Stop**, and then tap **Stop** again. To continue with the scan, tap **Continue**.

7. After the scan is complete, tap **OK**:

Protocol step completed OK

20180131\_SL\_XH

Sample: Jurkat

Protocol: Hemocytometer

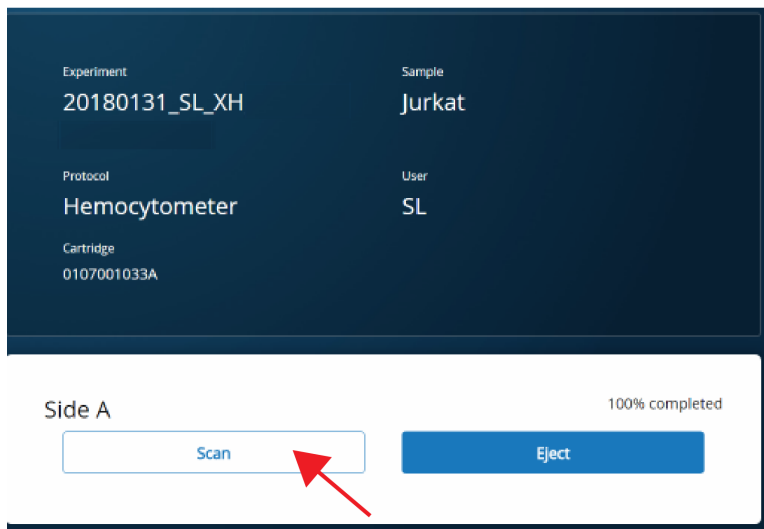
User: SL

Cartridge: 0107001033A

**Side A** 100% completed

Scan Eject

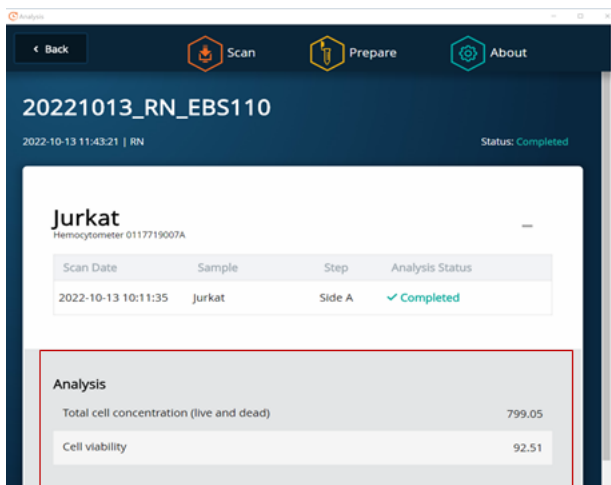
8. Scan the other side of the hemocytometer when necessary, or eject it:
  - Scan the other side: Tap **Scan**, enter a new sample name, and then repeat **steps 6–7**. The hemocytometer remains in the scanner and saves time by not ejecting the tray. For example:

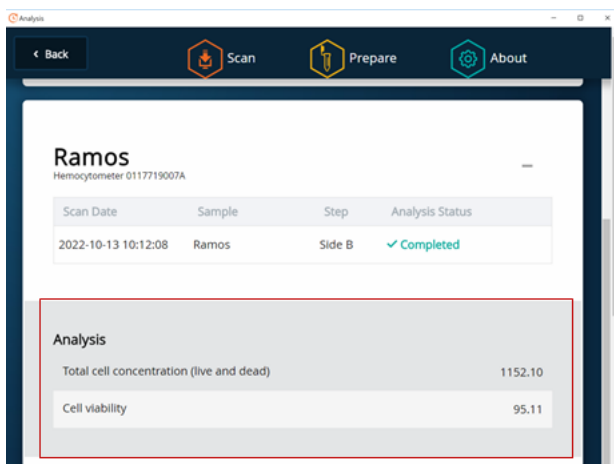


- Eject the hemocytometer: Tap **Eject**. The Hemocytometer Adapter is ejected from the scanner, and the remove cartridge screen displays.

**Note:** The analysis runs in the background. For example, the cell scan step for Jurkat and Ramos cells is complete. See **Step 11**.

9. Remove the Hemocytometer Adapter from the tray, and then tap **Done** to retract the tray and return to the main menu.
10. On the main menu, tap **Analysis**, and then tap the experiment. If no results are displayed, tap **Analyze**.
11. View the **total cell concentration** and **cell viability** on the results screen. For example, cell concentrations and viabilities are displayed for Jurkat and Ramos samples:





**Note:** If no value could be calculated, then **NaN** (not a number) is displayed. See [Troubleshooting](#) on page 50.

12. Proceed as follows:
  - If the cell concentration is  $\leq 1,000$  cells/ $\mu\text{L}$ , proceed to **step 14**.
  - If the cell concentration is  $> 1,000$  cells/ $\mu\text{L}$ , dilute the cell suspension in cold Sample Buffer to  $\sim 200$ – $800$  cells/ $\mu\text{L}$ . Recount the cells in the hemocytometer following **steps 1–11**, and then proceed to **step 13**.
13. Tap **Prepare** at the top of the results screen to display the Samples Calculator screen.
14. Dispose of the hemocytometer according to local safety regulations. See "Preparing a cell suspension and loading cells in the BD Rhapsody™ Cartridge," on [page 32](#).

**Note:** Minimize the time between cell pooling and single-cell capture.

## Preparing a cell suspension and 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 19.
- 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. 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) <sup>a,b</sup>	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%

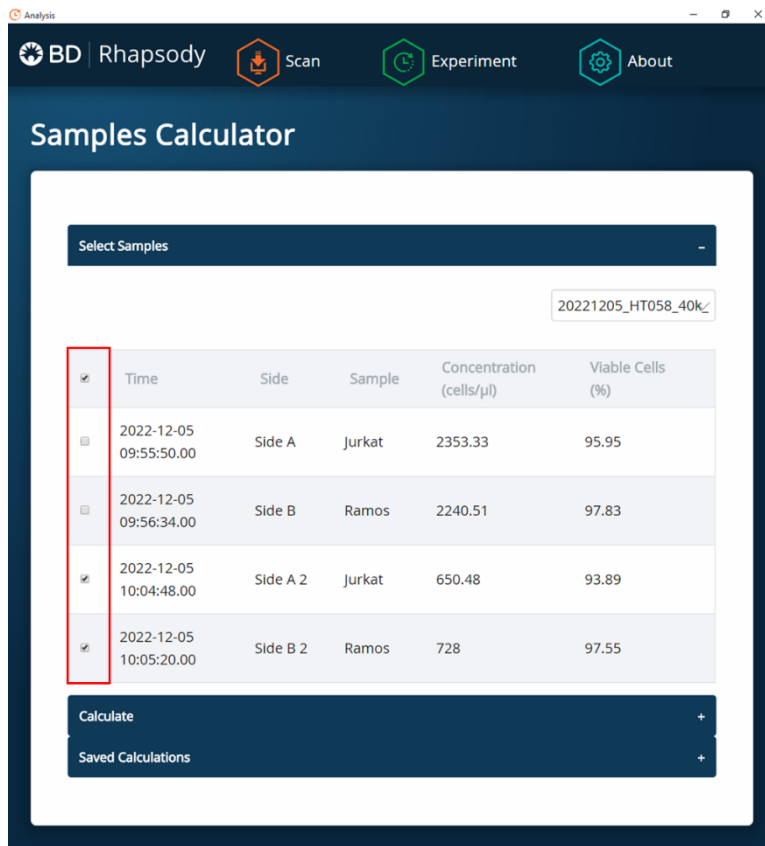
**Estimated multiplet rate based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads (continued)**

Number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads (target) <sup>a,b</sup>	Estimated multiplet rate
25000	4.6%
30000	5.5%
35000	6.4%
40000	7.3%
45000	8.2%
50000	9.1%
55000	10.0%

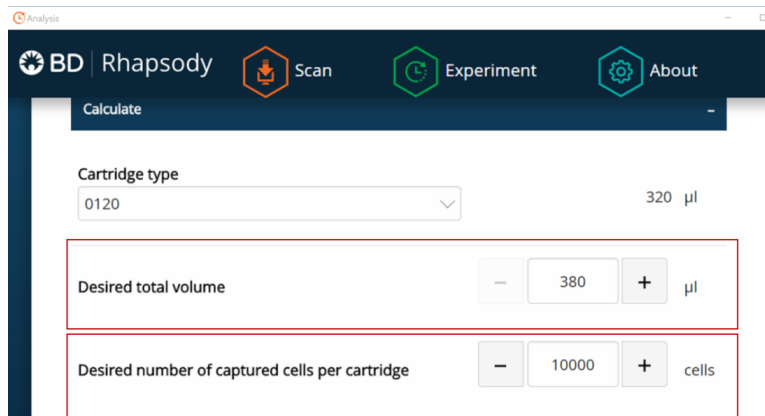
a. The number of cells sequenced might be less than the number of cells captured due to bead loss during handling, panel choice, and sample composition. The validated range of cells sequenced is 100–55,000 cells dependent on the library preparation.

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. According to the number of captured cells chosen in **step 1**, use the Samples Calculator on the scanner to obtain the volume(s) of cell stock and volume of cold Sample Buffer to prepare a cell suspension of 380 µL for loading into a BD Rhapsody™ 8-Lane Cartridge.
3. In the BD Rhapsody™ Scanner software, navigate to the **Analysis** screen, and proceed to the next step. If you are on the results screen that displays the viability and concentration of cells, tap **Prepare** at the top of the screen, and skip to **step c**.
  - a. On the Analysis screen, tap the experiment.
  - b. On the results screen, tap **Prepare**.
  - c. On the Samples Calculator screen, select the experiment from the drop-down menu.
  - d. You can search an experiment on the current screen by entering keywords in the search box.
  - e. If necessary, tap the checkboxes to clear samples individually or tap the checkbox in the header column to clear them all at once. For example, two samples are selected.



- f. Tap **Calculate**.
- g. Select the cartridge type from the drop-down menu. The cartridge type is the first four digits on the barcode label of the cartridge.
- h. Enter the **Desired total volume** and the **Desired number of captured cells**. For example, a desired total volume of 380 μL of a mixture of two samples and 10,000 cells captured are entered.



- i. If necessary, enter the relative amount of each sample below the desired number of captured cells. For example, equal amounts of two samples are entered:

Sample	Time	Concentration (cells/ $\mu$ l)	Viable Cells (%)	Relative Amounts
Jurkat	2022-12-05 10:04:48.00	650.48	93.89	- 1 +
Ramos	2022-12-05 10:05:20.00	728	97.55	- 1 +

- j. Obtain the calculated cell stock and buffer volumes to prepare the cell suspension for loading into the cartridge.

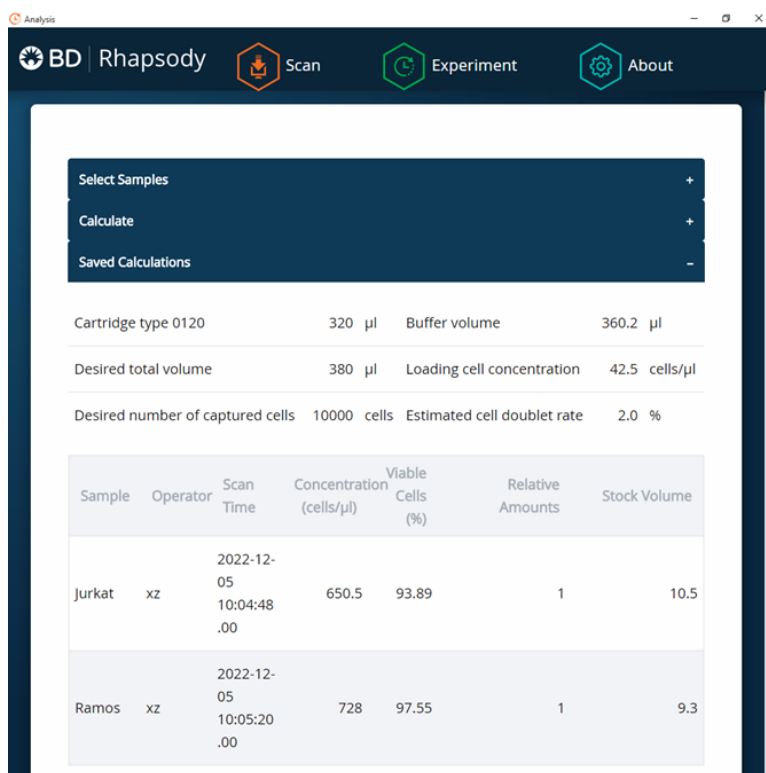
For example, two cell stock volumes and the buffer volume are calculated:

Results	
Sample	Stock Volume
Jurkat	10.5 $\mu$ l
Ramos	9.3 $\mu$ l
Buffer volume	360.2 $\mu$ l
Loading cell concentration	42.5 cells/ $\mu$ l
Estimated cell doublet rate	2.0%

Save

Saved Calculations +

- k. Tap **Save**. You can view the calculation by clicking the **Rhapsody Data** shortcut on the scanner screen and navigating to the appropriate folder.
- l. (Optional) Click **Saved Calculations**. You can click saved calculations at any time. For example, the saved calculations from one experiment with two samples are displayed.



#### 4. Prepare the cell suspension.

Stock cell volume or cell number	Cell suspension preparation
Calculated Stock Volume is <380 µL.	In a new 1.5-mL LoBind tube, prepare the cell suspension in cold Sample Buffer according to the volumes obtained in <b>step 3</b> .
<ul style="list-style-type: none"> <li>Calculated Stock Volume <math>\geq</math>380 µL, or</li> <li>Buffer volume is negative, or</li> <li>Cell concentration too low warning is displayed.</li> </ul>	Use 380 µL of the cold cell suspension for cartridge cell loading. <sup>a</sup>
<p>a. 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. For example, if the Samples Calculator specifies mixing 400 µL of Jurkat cells and 30 µL of Ramos cells, mix 350 µL of Jurkat cells with 30 µL of Ramos cells.</p>	

**Note:** Ensure the stock solution is well suspended by gently pipetting cells up and down before transferring the appropriate volume for dilution. The volumes are given for a single lane in the cartridge. Multiply (x8) to prepare the volumes for the entire 8-lane cartridge if loading the same sample.

- Transfer each sample of cell suspension to a 96-deep well plate for multiple lane loading. Keep on ice.
- 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.
- Change the mode of the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX) to **Load**.
- Before loading cells, use a manual pipette to gently pipet the cell suspension up and down to mix.
- Using the BD Rhapsody™ P8xP1200µL Pipette – HTX (or BD Rhapsody™ P1200µL Pipette – HTX), press the pipette button to aspirate 320 µL of the cell suspension.

10. Insert the tip(s) of the pipette perpendicular to the port, seal the pipette tip against the gasket, and then dispense 320  $\mu\text{L}$  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.

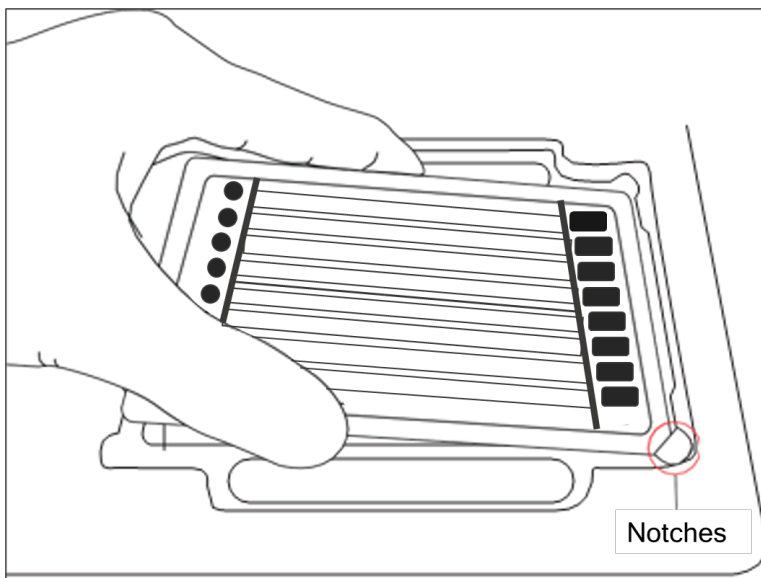
11. Incubate the cells by one of these methods:
  - On the BD Rhapsody™ HT Xpress System: Leave the cartridge with loaded cells on the tray at room temperature (15–25 °C) for 8 minutes. During incubation on the laboratory bench, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See [Preparing BD Rhapsody™ Enhanced Cell Capture Beads on page 42](#). After preparing the BD Rhapsody™ Enhanced Cell Capture Beads, proceed to [Imaging cells in a cartridge on page 38](#), or
  - In the scanner: Proceed immediately to [Imaging cells in a cartridge on page 38](#) to install the cartridge on the BD Rhapsody™ Scanner and enter a 8-minute run delay.

## Imaging cells in a cartridge

1. Navigate to the **Scan** application. The tray door of the scanner opens automatically, and the tray is ejected.

**Note:** If the tray is not ejected, tap the eject button in the upper right of the cartridge insertion screen.

2. Push the cartridge into the far end of the BD Rhapsody™ HT Xpress System 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.
3. If necessary, wipe condensation from the top surface of the cartridge with a lint-free wipe to ensure optimal scanning.
4. Place the cartridge on the scanner tray so that the cartridge and tray notches match and the barcode faces toward the front of the instrument.



### Notes:

- The scanner displays an alert if the cartridge is in the wrong orientation.
  - You can manually enter the barcode in the application, if necessary.
5. Tap **Continue**. The tray retracts, the door closes, and the scanner displays the experiment **workflow** screen.

6. Select from the drop-down menu or enter the experiment name, type the sample names for each lane used and press enter, select the protocol, and type in the username.

BD | Rhapsody

Experiment: experiment name... Cartridge: 01200051234

User: your name here...

Protocol: protocol...

Select lanes for scanning and enter sample name for each lane

A: Tap to select and enter sample name

B: Tap to select and enter sample name

C: Tap to select and enter sample name

D: Tap to select and enter sample name

E: Tap to select and enter sample name

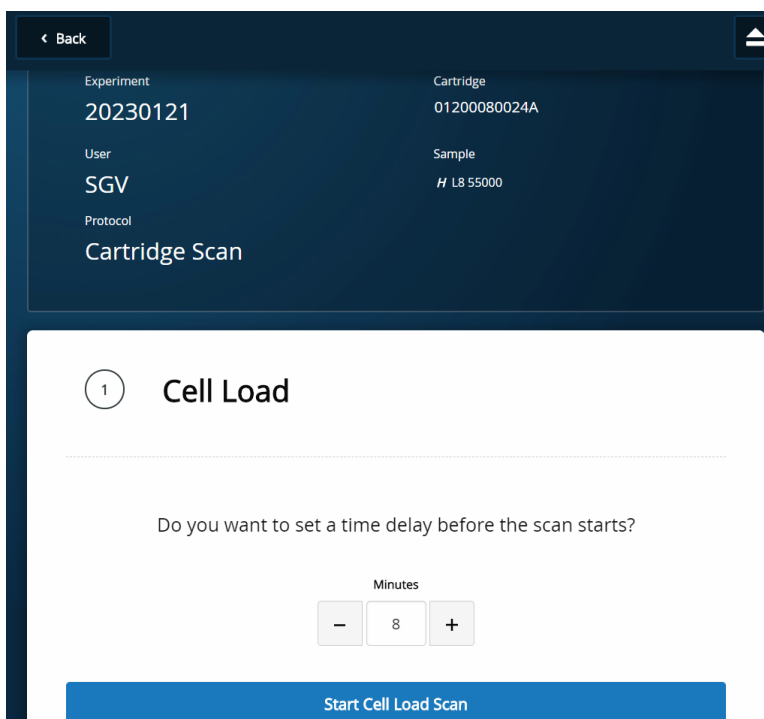
F: Tap to select and enter sample name

G: Tap to select and enter sample name

H: Tap to select and enter sample name

Continue

7. Tap **Cell Load**.



8. Proceed according to incubation location:
  - BD Rhapsody™ HT Xpress System (8 minute incubation completed): Proceed to **step 9**.
  - Incubation in scanner: Enter the appropriate time delay to delay the start of the scan. For example, 8 minutes or whatever number of minutes is left for incubation, and incubate the cartridge in the scanner by proceeding to **step 9**. During incubation in the scanner, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See [Preparing BD Rhapsody™ Enhanced Cell Capture Beads on page 42](#). After preparing the BD Rhapsody™ Enhanced Cell Capture Beads, proceed to **step 10**.
9. Tap **Start Cell Load Scan**. The tray retracts into the scanner, and the door closes. If entered, the 8-minute countdown time displays in the Cell Load window. The scan proceeds after the countdown completes. The scan takes an additional 11 minutes to complete for an entire BD Rhapsody™ 8-Lane Cartridge.
 

**Note:** To stop the scan, tap **Stop**, and then tap **Stop** again. A scan can be repeated on a lane that was stopped in the middle of a scan. Once the scanner has finished scanning a lane, re-scanning of that lane is not possible. To continue with the current scan, tap **Continue**.
10. After the scan is complete, a green box “Protocol Scan Completed” is displayed. Tap **OK**, and then **Eject**. The cartridge is ejected from the scanner, and the remove cartridge screen is displayed.

**Note:** The analysis runs in the background. **Do not close the analysis app before or during the run.**





11. To confirm that the analysis is running, navigate to the **Analysis** screen, and then tap the experiment to view the ongoing analysis on the results screen. Upon completion, the results are listed.

## H L8 55000

Cartridge 01200080024A

Scan Date	Sample	Step	Analysis Status
2023-01-21 12:43:19	L8 55000	Cell Load	✓ Completed

**Note:** If the analysis is not running, tap **Analyze**. To re-analyze a scan, select the lane, and tap the Analysis Status for the scan, and then tap **Re-Analyze**.

## Preparing BD Rhapsody™ Enhanced Cell Capture Beads

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### 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.

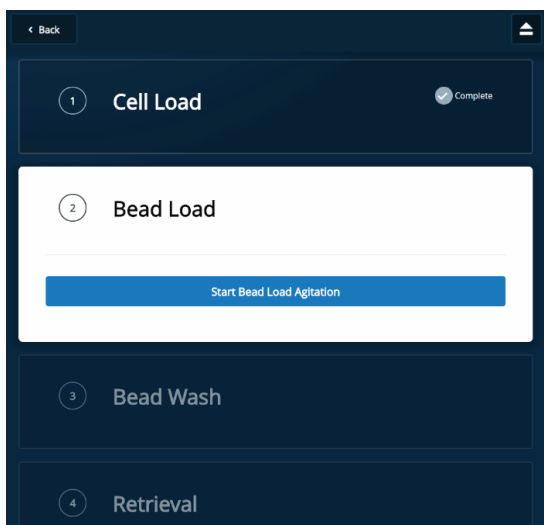
1. Place the tube with BD Rhapsody™ Enhanced Cell Capture Beads on the magnet for 1 minute.
2. Carefully remove and appropriately discard the storage buffer without disturbing the beads and while leaving the tube on the magnet.
3. Remove the tube from the magnet, and then pipet 380  $\mu\text{L}$  of 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 until Cell Load scan is complete.
7. After the Cell Load scan, and after you have confirmed that the analysis is running, proceed to [Loading BD Rhapsody™ Enhanced Cell Capture Beads and imaging on page 42](#).

## Loading BD Rhapsody™ Enhanced Cell Capture Beads and imaging

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1. Return the cartridge to the tray of the BD Rhapsody™ HT Xpress System.
2. Change the mode of the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) to **Prime/Wash**.
3. Load the cartridge with 380  $\mu\text{L}$  of air using the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) in **Prime/Wash** mode.
4. Change the mode of the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) to **Mix** mode.
5. Use a BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  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.
6. With new pipette tips, set the pipette to **Load** mode.
7. 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.
8. Using the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) in **Load** mode, immediately load the cartridge with 320  $\mu\text{L}$  of beads.
9. Let the beads settle in the cartridge on the tray at room temperature (15–25 °C) for 3 minutes.

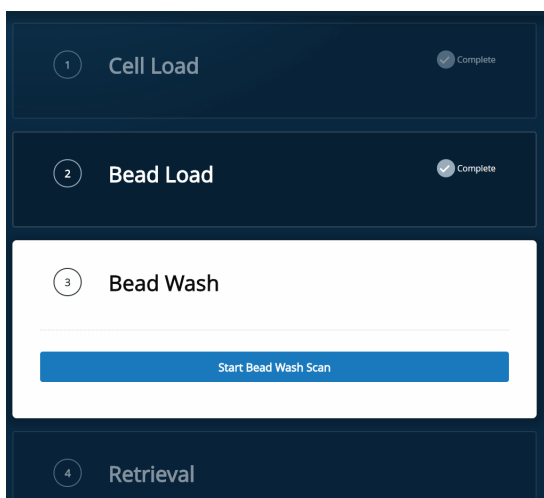
10. Place the BD Rhapsody™ 8-Lane Cartridge in the BD Rhapsody™ Scanner and perform the 2 minute Bead Agitation Step.



11. After Bead Agitation is complete, tap **OK** and **Eject**. Remove the cartridge from the scanner.
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 15–16** once for a total of two washes.
18. Image the cartridge with the scanner (**Bead Wash** step).



## 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  $-20\text{ }^{\circ}\text{C}$ .

1. Add 75.0  $\mu\text{L}$  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

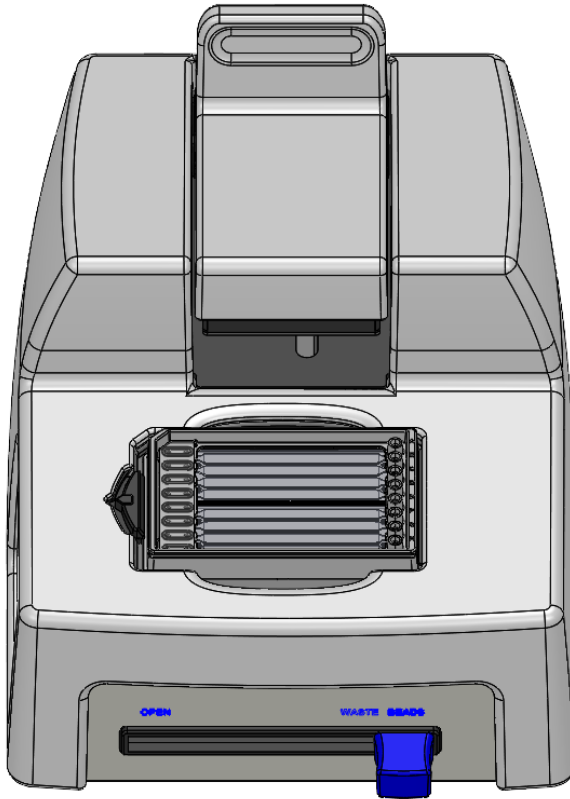
3. Return the cartridge to the tray of the BD Rhapsody™ HT Xpress System.
4. Set the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) to **Lysis** mode.
5. Load the cartridge with 280  $\mu\text{L}$  of Lysis Buffer with DTT using the BD Rhapsody™ P8xP1200 $\mu\text{L}$  Pipette – HTX (or BD Rhapsody™ P1200 $\mu\text{L}$  Pipette – HTX) in **Lysis** mode.
6. Incubate at room temperature (15–25  $^{\circ}\text{C}$ ) for 2 minutes.

Maintain the recommended lysis time for best performance.

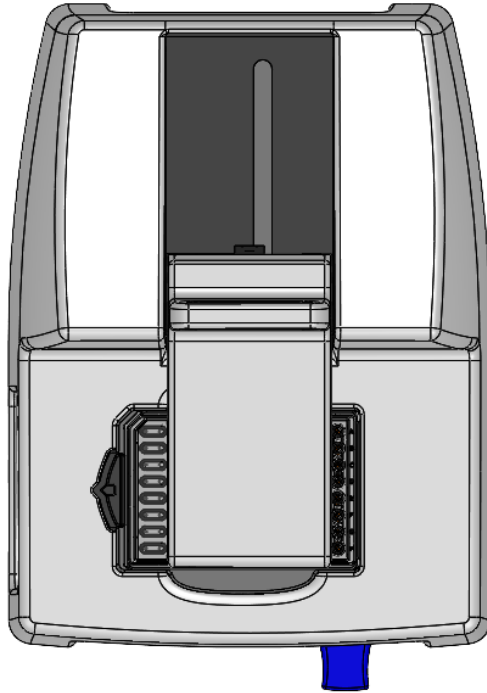
**Note:** Before retrieval, remove extra buffer that has pooled at the inlet with a P20 pipette to minimize overflow. Aspirate at an angle to avoid accidental aspiration of buffer volume in the microwell array.

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

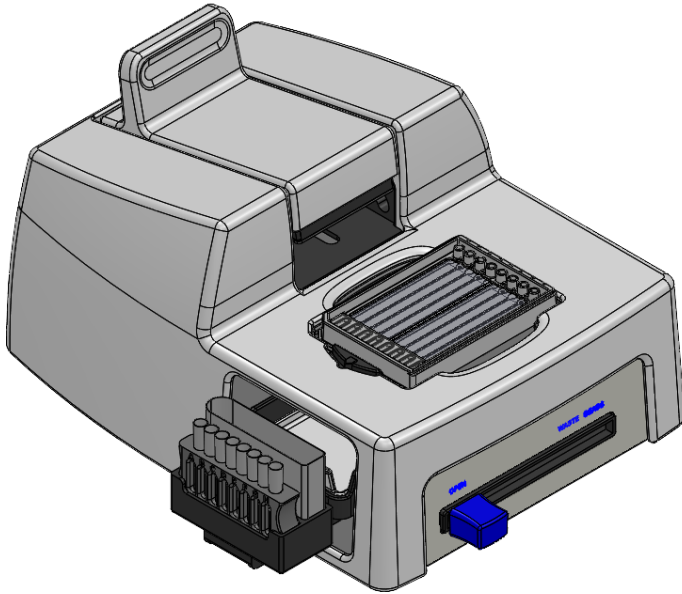
1. Confirm that a cluster tube was placed in the BD Rhapsody™ HT Xpress System drawer for bead retrieval. Label the tubes appropriately.
2. Move the front slider to **BEADS** on the BD Rhapsody™ HT Xpress System.



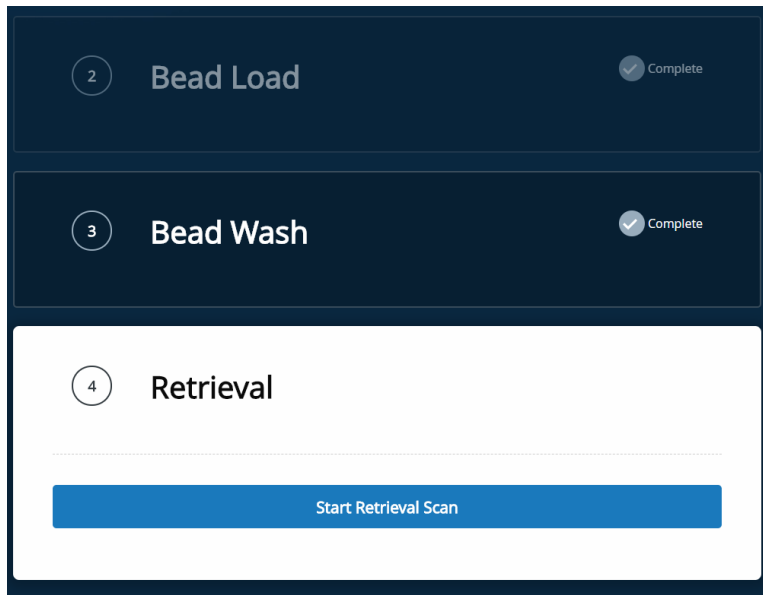
3. Gently pull the top **RETRIEVAL** slider onto the BD Rhapsody™ 8-Lane Cartridge (ACTIVE). Make sure that the Retrieval magnet is in contact with the BD Rhapsody™ 8-Lane Cartridge.



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. Image the cartridge with the scanner: **Retrieval**. Note that it is normal that one or two drops of residue liquid is observed on the loading station. Blot with a lint-free wipe.



14. After the scan is complete, tap **OK** and **Eject**. Remove the cartridge from the scanner and tap **DONE**. After the scan, confirm the analysis is running.
15. Immediately proceed to [Washing BD Rhapsody™ Enhanced Cell Capture Beads on page 48](#).
16. Keep partially used cartridges on a flat surface while [Washing BD Rhapsody™ Enhanced Cell Capture Beads on page 48](#).

17. Perform "Washing used lanes and BD Rhapsody™ 8-Lane Cartridge storage procedure" during cDNA synthesis. See the *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol*.
18. 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 63](#).

19. Appropriately dispose of the Lysis Buffer with DTT.
20. 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*.
21. Clean partially used cartridges following the procedures in "Cartridge cleaning and storage procedure." See the *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol*.

## Washing BD Rhapsody™ Enhanced Cell Capture Beads

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### 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.**

## 6. Troubleshooting

### Scanning troubleshooting

The following table describes possible problems and recommended solutions for scanning issues.

#### Scanning troubleshooting

Observation	Possible causes	Recommended solutions
Incorrect alignment.	Cartridge or hemocytometer not properly inserted on BD Rhapsody™ HT Xpress System tray.	Ensure that the notched corner of the cartridge or hemocytometer is aligned with the notch of the tray, and the barcode is facing toward the front of the instrument.
Tray not ejected from BD Rhapsody™ Scanner.	Cartridge in incorrect orientation or no cartridge.	Tap the eject button at the right top corner of the cartridge insertion screen. Reinsert the cartridge on the tray.
BD Rhapsody™ Scanner not analyzing all images.	Masked or incompletely processed wells.	The software automatically corrects for masked or incompletely processed wells. No manual extrapolation is necessary.
Persistent scanning artifacts.  Artifacts might include apparent cells in an empty hemocytometer, consistently low capture rate warnings, auto-exposure errors, and dirty optics cover.	Dirty optics.	Contact BD Biosciences technical support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .

## Cartridge loading troubleshooting

The following table describes possible problems and recommended solutions for BD Rhapsody™ Cartridge issues that arise during image analysis. Also see [BD Rhapsody™ Scanner metrics on page 57](#).

### Cartridge loading troubleshooting

Observation	Possible causes	Recommended solutions
Number of cells captured too high or cell doublet rate too high (out of range).	Too many cells loaded.	<ul style="list-style-type: none"> <li>Confirm that the calculation of cell dilution is correct.</li> <li>Reduce the number of cells loaded in the cartridge.</li> </ul>
	Improper counting with hemocytometer.	<ul style="list-style-type: none"> <li>Recount cells with the BD Rhapsody™ Scanner.</li> <li>Follow <a href="#">Processing cells with BD Rhapsody™ HT Single-Cell Analysis System on page 26</a>.</li> </ul>
	Incorrect calculation of cell concentration.	<ul style="list-style-type: none"> <li>Use the BD Rhapsody™ Scanner for cell counting and analysis.</li> <li>Use the Analysis app Sample Calculator for preparing cell suspensions.</li> </ul>
Scanner unable to detect red stained cells (error message appears).	Error in software/unwanted interruptions by the user.	<ul style="list-style-type: none"> <li>Re-scan the hemocytometer with the BD Rhapsody™ Scanner.</li> </ul> <p>If the problem persists, contact BD Biosciences technical support at <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a>.</p>
No cell viability is reported.	Scanner unable to detect red stained cells.	<ul style="list-style-type: none"> <li>Tap <b>Reanalyze</b> in the Analysis Status to calculate cell viability again.</li> <li>Re-scan the hemocytometer with the BD Rhapsody™ Scanner.</li> </ul> <p>If the problem persists, contact BD Biosciences technical support at <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a>.</p>
Number of cells captured lower than anticipated.	Incorrect calculation of cell concentration.	<ul style="list-style-type: none"> <li>Use the BD Rhapsody™ Scanner for cell counting and analysis.</li> <li>Use the Analysis app Sample Calculator for preparing cell suspensions.</li> </ul>
	Cell size larger than recommended range.	<ul style="list-style-type: none"> <li>BD Rhapsody™ Enhanced Cell Capture Beads efficiency is reduced in the BD Rhapsody™ Scanner if the cell diameter is &gt;20 µm.</li> </ul>
	Insufficient incubation after cell loading.	<ul style="list-style-type: none"> <li>Make sure incubation time after cell loading is 8 minutes and not less.</li> </ul>

## Cartridge loading troubleshooting (continued)

Observation	Possible causes	Recommended solutions
Bead loading density out of range.	Insufficient number of BD Rhapsody™ Enhanced Cell Capture Beads loaded.	<ul style="list-style-type: none"> <li>• Ensure that all of the beads are pelleted before removing storage buffer and are resuspended in Sample Buffer.</li> <li>• Use the <b>Load</b> mode on the pipette.</li> <li>• Ensure the beads are well suspended before loading into the cartridge.</li> </ul>
Image analysis fails at Bead Wash, indicating insufficient removal of excess BD Rhapsody™ Enhanced Cell Capture Beads.	Pipette mode incorrect.	<ul style="list-style-type: none"> <li>• Ensure that the correct pipette mode is used for the step.</li> </ul>
	Pipette step missed.	<ul style="list-style-type: none"> <li>• Review protocol, and repeat, if necessary.</li> </ul>
Cell retention out of range, indicating cell loss during cartridge workflow.	Pipette mode incorrect.	<ul style="list-style-type: none"> <li>• Ensure that the correct pipette mode is used for the step.</li> </ul>
	Cell viability low.	<ul style="list-style-type: none"> <li>• Use cells of high viability.</li> </ul>
Bead retrieval efficiency out of range, indicating poor retrieval.	Retrieval (top) magnet not positioned properly.	<ul style="list-style-type: none"> <li>• Check magnet position.</li> </ul>
	Pipette mode incorrect.	<ul style="list-style-type: none"> <li>• Ensure that the correct pipette mode is used for the step.</li> </ul>
Retrieval Scan is aborted before scan completion of a particular lane in the cartridge.	Unwanted interruptions by the user.	<ul style="list-style-type: none"> <li>• Do not click the eject button for the cartridge if the Retrieval scan is interrupted. Instead, click <b>Continue Scan</b>, then click <b>Resume scanning incomplete lanes</b> to re-scan the lanes selected for the Retrieval step.</li> </ul>
Image analysis does not start.	Software does not initiate analysis after a scan.	<ol style="list-style-type: none"> <li>1. On the BD Rhapsody™ Scanner main menu, tap <b>Analysis</b>.</li> <li>2. Tap the experiment.</li> <li>3. Choose the appropriate sample lane.</li> <li>4. Tap <b>Analyze/Reanalyze</b> on the Analysis Status to start the analysis. You can analyze multiple scan steps at the same time.</li> </ol>
Dropped the cartridge or hit it against object.	Various.	<ul style="list-style-type: none"> <li>• If the cartridge was dropped, we recommend using a new cartridge. Otherwise, carefully review the imaging metrics before proceeding.</li> <li>• If the cartridge was struck, proceed at your own risk, and carefully review the imaging metrics before proceeding.</li> </ul>

**Cartridge loading troubleshooting (continued)**

Observation	Possible causes	Recommended solutions
Air bubble in cartridge.	Air bubble present in pipette tip while dispensing buffer.	<ul style="list-style-type: none"> <li>• Confirm that there is an air bubble in the cartridge by examining an image of the cartridge.</li> <li>• Ensure that the pipette tip contains only buffer, dispense the buffer into the cartridge and confirm no air bubble remains.</li> <li>• If air bubbles are present, the user should not proceed with the scan as the scanner metrics will be compromised.</li> </ul>
	Re-used pipette tip.	<ul style="list-style-type: none"> <li>• Use a new pipette tip at every pipetting step.</li> </ul>
Image analysis fails.	Contact BD Biosciences technical support at <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .	
Installation or mechanical issues.	See the <i>BD Rhapsody™ HT Xpress Installation and Maintenance Guide</i> .	

## BD Rhapsody™ Scanner software messages

The following table lists error and warning messages that you might encounter while using the BD Rhapsody™ Scanner software.

If the recommended solutions do not resolve the problem, contact BD Biosciences tech support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).

### Error messages

Error messages require you to perform an action, according to a displayed dialog, before you can proceed.

#### Error messages

Error message	Possible causes	Recommended solutions
“Failed to open door. Ensure nothing is blocking the door.”	Obstruction.	Remove obstruction, and retry opening the scanner cartridge door.
“Failed to open door.”	Various. Not an obstruction.	Restart scanner.
“Failed to close door. Ensure nothing is blocking the door.”	Obstruction.	Remove obstruction, and retry closing the scanner cartridge door.
“Failed to close door.”	Various. Not an obstruction.	Restart the scanner.
“Obstruction detected while ejecting.”	Obstruction or calibration update required.	Remove obstruction, and retry ejecting the cartridge.
“Obstruction detected while retracting stage.”	Obstruction.	<ol style="list-style-type: none"> <li>1. Remove obstruction, and retry retracting cartridge into the scanner.</li> <li>2. Click <b>Cancel</b>.</li> <li>3. Restart the scanner.</li> </ol>
“Available disk space is extremely low.”	Low disk space.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Failed to initialize...”	Various.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Requested move not safe.”	Various.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Unknown cartridge barcode.”	Unsupported barcode scanned.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Failed to read file.”	Various.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Cartridge alignment feature not found.”	<ul style="list-style-type: none"> <li>• Incorrect cartridge.</li> <li>• Light failed.</li> </ul>	Rescan the cartridge.
“Failed to start analysis messaging because: Exception of type 'NetMQ.Address Already In Use Exception' was thrown.”	The last time Analysis software was run, it failed to shut down completely.	Restart the computer.

## Warnings

Warnings display in the Warnings box on the scanner display and are mostly informative messages that require no action from you.

### Warning messages

Warning message	Possible causes	Recommended solutions
“Barcode scan failed. Please make sure the cartridge is inserted and aligned correctly.”	Barcode not read.	<ol style="list-style-type: none"> <li>1. Reinsert the cartridge, and rescan the cartridge.</li> <li>2. The barcode can be manually entered.</li> <li>3. If necessary, restart the scanner.</li> </ol>
“Available disk space is running low.”	Limited disk space.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“The protocol was not found.”	Protocol missing.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Auto-exposure is not configured for image type. Using default value instead.”	Method not found.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Auto-exposure resulted in images darker than normal.”	<ul style="list-style-type: none"> <li>• No cell in image tile.</li> <li>• Light failed.</li> </ul>	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Auto-exposure resulted in images brighter than normal.”	Various.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Auto-exposure adjustment unsuccessful. Using nearest value.”	Various.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“Attempted to use exposure time greater than maximum. Using the maximum instead.”	Light failed.	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .
“No alignment marks found. Continuing without cartridge specific calibration.”	Cartridge misaligned/Cartridge defects.	Reinsert cartridge, and rescan.
“Not enough local alignment marks detected.”	<ul style="list-style-type: none"> <li>• Bead is covering an alignment mark.</li> <li>• Bad image.</li> </ul>	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> .

**Warning messages (continued)**

Warning message	Possible causes	Recommended solutions
<p>“Not enough local fiducials detected.”</p>	<p>Image is out of focus, or bubbles, or clumps of beads have accumulated in the cartridge. After reprocessing with Analysis, review the number of tiles processed in the cartridge results CSV file located in the Public Documents\Rhapsody Data folder of your computer.</p> <p>If the number of tiles is the same as the total number in the cartridge results CSV file, reprocessing is successful. Otherwise, results are extrapolated.</p>	<p>Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a>.</p>

If there are any other errors or warnings, please contact BD tech support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) or 1.877.232.8995.



## 7. BD Rhapsody™ Scanner metrics

### Image analysis metrics

#### Understanding analysis metrics

The chart lists the steps and metrics measured during image analysis of the hemocytometer or BD Rhapsody™ 8-Lane Cartridge.

#### Understanding analysis metrics

Step	Metric	Definition	Use	Passing threshold
Hemocytometer	Total cell concentration (cell/μL).	Number of viable (Calcein AM-positive) and non-viable(DRAQ7-positive) cells/μL.	Calculates concentration of the cell suspension to determine the volume of cell suspension to use for loading onto the cartridge.	Value
Hemocytometer	Cell viability (%).	Percent viable (Calcein AM-positive) cells.	Assesses cell viability to determine if the cell sample meets the quality threshold.	Value
Cell Load	Number of wells with viable cells at Cell Load.	Number of wells containing viable (Calcein AM-positive) cells.	Provides a preliminary estimate of the number of wells with viable cells captured. <sup>a</sup>	–
Cell Load	Number of viable cells captured in wells at Cell Load.	Number of viable (Calcein AM-positive) cells captured in a well.	Provides a preliminary estimate of the total number of viable cells captured in the cartridge. <sup>b</sup>	–
Cell Load	Cell multiplet rate at Cell Load (%).	Number of wells containing multiple viable (Calcein AM-positive) cells versus the total number of wells containing one or more viable cells	Provides a measure of cell clumping.	–
Bead Wash	Number of wells with viable cells and a bead.	Total number of wells with one bead and ≥1 viable (Calcein AM-positive) cells.	Provides an estimate of the number of wells with viable cells captured with beads. <sup>c</sup>	Value
Bead Wash	Number of viable cells captured in wells with a bead.	Total number of viable (Calcein AM-positive) cells captured in a well with a bead.	Estimates the total number of viable cells captured on a BD Rhapsody™ Enhanced Cell Capture Bead at cell lysis. <sup>c</sup>	–

**Understanding analysis metrics (continued)**

Step	Metric	Definition	Use	Passing threshold
Bead Wash	Cell multiplet rated (%)	Number of wells containing one bead and multiple viable (Calcein AM-positive) cells versus the total number of wells containing one bead and one or more viable cells.	Provides an estimate of the occurrence of multiple cells captured by the same bead in a well.	Value
Bead Wash	Bead loading efficiency (%)	Percent wells with one bead.	Indicates if the cartridge is significantly underloaded with beads.	≥80%
Bead Wash	Cell retention rate (%)	Number of viable (Calcein AM-positive) cells captured in wells prior to lysis versus number of viable cells captured in wells prior to bead loading.	Indicates if a significant number of cells initially loaded into wells are lost or died during the workflow prior to lysis.	≥60%
Retrieval	Bead retrieval efficiency (%)	Percent of wells with beads removed.	Indicates if the number of beads retrieved is significantly lower than expected.	≥90%
<p>a. The number of wells with viable cells is less than the total number of viable cells captured in wells because of wells containing cell multiplets.</p> <p>b. <math>BD\ Rhapsody^{TM}\ Enhanced\ Cell\ Capture\ Beads\ cell\ capture\ efficiency\ at\ Cell\ Load\ (\%) = \frac{\text{Number of viable cells captured in well}}{\text{Cell Load/Total number of viable cells imaged}} * 100.</math></p> <p>c. The metric, the number of wells with viable cells and a bead, is less than the metric, the number of viable cells captured in wells with a bead, due to wells containing cell multiplets. Multiple cells captured with one bead will appear as one cell in sequencing data. Because of this, the appropriate metric to use for an estimation of the number of cells that could be recovered in sequencing is the metric, number of wells with viable cells and a bead.</p> <p>d. The estimated multiplet rate is calculated by Poisson distribution using the number of cells loaded into the cartridge. See <a href="#">Processing cells with BD Rhapsody™ HT Single-Cell Analysis System on page 26</a>. The cell multiplet rate reported by the BD Rhapsody™ Scanner is determined by imaging. If the cell multiplet rate &gt;&gt; estimated multiplet rate, this might indicate cell clumping.</p>				

**Viewing image analysis metrics**

To view image analysis metrics in a spreadsheet, click the Rhapsody Data shortcut on the BD Rhapsody™ Scanner screen.

## Reviewing the analysis metrics

1. Navigate to the Analysis application.
2. Tap the experiment to ensure that the analyses are completed.

For example:

**A 1**  
Cartridge 01200140080A

Scan Date	Sample	Step	Analysis Status
2023-01-18 10:57:45	1	Cell Load	✓ Completed
2023-01-18 11:16:15	1	Bead Wash	✓ Completed
2023-01-18 11:28:23	1	Retrieval	✓ Completed

3. Review the analysis metrics. For PASS criteria, see [Image analysis metrics on page 57](#). For example:

Analysis	
Number of wells with viable cells at cell load	38620
Cell multiplet rate at cell load	9.0 %
Number of wells with viable cells and a bead	36820
Cell multiplet rate	9.3 %
Bead loading efficiency	✓ PASS
Excess bead rate	✓ PASS
Cell retention rate	✓ PASS
Bead retrieval efficiency	✓ PASS

4. (Optional) View quantitative analysis metrics for each experiment in a spreadsheet. For example, the .csv files from the analysis of the Jurkat and Ramos cells are displayed:

**Note:** To set up the shortcut to the Rhapsody Data folder in Quick Access, see [BD Rhapsody™ Scanner software on page 15](#).

- [\(1\) Analysis metrics on page 60](#)
- [\(2\) Cell count on page 60](#)
- [\(3\) Sample result on page 61](#)

## (1) Analysis metrics

Analysis	
Number of wells with viable cells at cell load	38620
Cell multiplet rate at cell load	9.0 %
Number of wells with viable cells and a bead	36820
Cell multiplet rate	9.3 %
Bead loading efficiency	✓ PASS
Excess bead rate	✓ PASS
Cell retention rate	✓ PASS
Bead retrieval efficiency	✓ PASS

## (2) Cell count

Analysis software version	2.0.0.196
Experiment	20230121
Sample	Jurkat
Barcode	01200130030A
Protocol step	Side A
Scan System ID	R-NHB227119UV00
Operator	MP
Scan start date and time	01/21/2023 12:17
Analysis last updated	01/21/2023 12:17
Total cell concentration (live and dead)	553.71
Cell viability	94.98

Analysis software version	2.0.0.196
Experiment	20230121
Sample	Ramos
Barcode	01200130030A
Protocol step	Side B
Scan System ID	R-NHB227119UV00
Operator	MP
Scan start date and time	1/21/2023 12:25
Analysis last updated	1/21/2023 12:25
Total cell concentration (live and dead)	575.55
Cell viability	94.65

**(3) Sample result**

Experiment	20230121					
Cartridge type	120					
Cartridge volume (µL)	320					
Total volume to prepare (µL)	380					
Desired number of captured cells	20000					
Sample buffer volume (µL)	308.2					
Loading cell concentration	85					
Cell doublet rate (%)	4.1					
Sample	Operator	Scan Date and Time	Concentration	Viable Cells	Relative Amount	Stock Volume (µL)
Jurkat	MP	15:20.1	553.71	94.98	1	26.6
Ramos	MP	19:25.7	575.55	94.65	1	45.2

## 8. Updating BD Rhapsody™ Scanner software

We recommend updating the scanner software by navigating the installation with keyboard and mouse, and contacting BD technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com). For recommended peripherals, see a BD Rhapsody™ user guide.

## 9. Safety

### General safety and limitations

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For instrument safety, see the *BD Rhapsody™ HT Single-Cell Analysis 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](https://scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library).

### Chemical safety

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#### 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™ 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	<ul style="list-style-type: none"> <li>• Frequency of Handling: Every BD Rhapsody™ experiment.</li> <li>• Content: Ethanol (11%), polymer micro particles (&lt;1%), cells (trace).</li> <li>• Main Risk Constituent: Cells (trace).</li> <li>• Collect and dispose of all waste in the Waste collection container using proper precautions and according to local safety regulations.</li> </ul>
Stream 2: BD Rhapsody™ Cartridge	<ul style="list-style-type: none"> <li>• Frequency of Handling: Every BD Rhapsody™ experiment.</li> <li>• Content: Polymer (99%), polymer micro particles (&lt;1%), Lysis Buffer (&lt;1%).</li> <li>• Main Risk Constituent(s): Lysis Buffer.</li> <li>• Collect and dispose of all used BD Rhapsody™ Cartridges using proper precautions and according to local safety regulations.</li> </ul>

### Physical safety

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See the *BD Rhapsody™ HT Single-Cell Analysis 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](https://scomix.bd.com/hc/en-us/categories/360000838932-Resource-Library).

## Instrument waste disposal

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Contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) before moving the BD Rhapsody™ Scanner or disposing of the BD Rhapsody™ HT Single-Cell Analysis System. For more information, see [Instrument technical support on page 6](#).



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