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

Copyrights

No part of this publication may be reproduced, transmitted, transcribed, stored in retrieval systems, or translated into any language or computer language, in any form or by any means: electronic, mechanical, magnetic, optical, chemical, manual, or otherwise, without prior written permission from BD.

The information in this guide is subject to change without notice. BD reserves the right to change its products and services at any time. Although this guide has been prepared with every precaution to ensure accuracy, BD assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information. BD welcomes customer input on corrections and suggestions for improvement.

Trademarks

BD, the BD Logo, BD Rhapsody and Pharmingen are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2022 BD. All rights reserved.

For US patents that may apply, see bd.com/patents.

Regulatory information

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

History

| Revision | Date | Change made |
|--|---------|---|
| Doc ID: 214062 Rev. 1.0 | 2018-07 | Initial release. |
| Doc ID: 214062 Rev. 2.0 23-21336-00 | 2019-02 | Added information about the BD™ Mouse Immune Single-Cell Multiplexing Kit |
| Doc ID: 214062 Rev. 3.0 23-21336-01 | 2019-08 | <ul style="list-style-type: none">— Updated analysis metrics, cell count, and sample result for BD Rhapsody™ Scanner.— Updated error and warning messages for BD Rhapsody™ Scanner software.— Added reference to BD Rhapsody™ Scanner Software Installation Guide (Part Number: 23- 20709-01) in Appendix C: Updating the BD Rhapsody™ Scanner software.— Updated BD Biosciences technical support Email address to scomix@bdscomix.bd.com.— Removed BD Rhapsody™ Training Cells Kit information from the Required kits and storage conditions section. |
| Doc ID: 214062 Rev. 4.0 23-21336-02 | 2020-07 | <ul style="list-style-type: none">— Updated European technical support phone number.— Updated catalog number for reverse transcriptase.— Recommended thermomixer instead of heat block for Exonuclease I inactivation. |

| Revision | Date | Change made |
|---|---------|---|
| Doc ID: 214062 Rev. 5.0 23-21336(03) | 2021-11 | <ul style="list-style-type: none"> — Removed library preparation references and associated kits. — Updated Cell Capture Bead reference to include the Enhanced Cell Bead with new Cat. No. — Removed kit component information and storage conditions from the Required kits and storage conditions section. — Removed information about BD Rhapsody™ Custom Panel. — Updated Consumable section to include only relevant information for the Cartridge Workflow. — Updated Equipment section to include only relevant information for the Cartridge Workflow. — Removed Software section. — Added a reference to how to perform reverse transcription for assays that profile BCR/TCR. — Moved the washing cell capture bead information to its own section preceding Performing reverse transcription on Cell Capture Beads section. — Added instructions to dissolve the precipitation in Lysis Buffer and DTT back to solution. — Removed all references to document Single-Cell Analysis Workflow with BD Rhapsody™ Systems. (Doc ID: 220524) |
| 23-21336(04) | 2022-11 | Updated for BD Rhapsody™ Enhanced Cell Capture Beads version 2.0. |

Contents

| | |
|--|-----------|
| 1. Introduction | 6 |
| About this guide | 6 |
| Intended use | 6 |
| Safety symbols | 7 |
| Safety data sheets | 7 |
| Instrument technical support | 7 |
| 2. Getting Started | 8 |
| BD Rhapsody™ cartridge workflow | 8 |
| Site requirements | 8 |
| Thermomixer settings | 9 |
| Thermal cycler setup | 9 |
| Pipette settings | 9 |
| BD Rhapsody™ Express instrument overview | 10 |
| BD Rhapsody™ Scanner overview | 11 |
| Quick guide to the BD Rhapsody™ Scanner software | 13 |
| Best practices | 15 |
| 3. Product information | 18 |
| Required kits | 18 |
| Required reagents | 18 |
| Required consumables | 19 |
| Required equipment | 20 |
| Suggested equipment | 21 |
| 4. Preparing the BD Rhapsody™ Cartridge | 22 |
| Priming the BD Rhapsody™ Cartridge | 22 |
| Treating the surface of the cartridge | 26 |
| 5. Processing cells with the BD Rhapsody™ Single-Cell Analysis system | 27 |
| Best practices | 27 |
| Staining cells with viability markers | 27 |
| Loading cells into the Hemocytometer Adapter | 28 |
| Counting cells with the BD Rhapsody™ Scanner | 29 |
| Preparing a cell suspension and loading cells in the BD Rhapsody™ Cartridge | 32 |
| Imaging cells in a cartridge | 36 |
| Preparing BD Rhapsody™ Enhanced Cell Capture Beads | 39 |
| Loading BD Rhapsody™ Enhanced Cell Capture Beads and imaging | 40 |
| Lysing cells and retrieving BD Rhapsody™ Enhanced Cell Capture Beads | 40 |
| Washing the BD Rhapsody™ Enhanced Cell Capture Beads | 43 |
| STOP! | 44 |

| | |
|--|-----------|
| Performing reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads | 44 |
| Treating the sample with Exonuclease I | 46 |
| 6. Troubleshooting | 48 |
| Scanning troubleshooting | 48 |
| Cartridge loading troubleshooting | 49 |
| BD Rhapsody™ Scanner software messages | 51 |
| 7. BD Rhapsody™ Scanner metrics | 54 |
| Image analysis metrics | 54 |
| Reviewing the analysis metrics | 56 |
| 8. Workflow with two BD Rhapsody™ Cartridges | 59 |
| Workflow with two cartridges | 59 |
| Best practices with a two-cartridge workflow | 59 |
| 9. Updating the BD Rhapsody™ Scanner software | 60 |
| 10. Safety | 61 |
| General safety and limitations | 61 |
| Chemical safety | 61 |
| Physical safety | 61 |
| Instrument waste disposal | 62 |

1. Introduction

About this guide

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

To use the BD Rhapsody™ Single-Cell Analysis system without the BD Rhapsody™ Scanner see the *BD Rhapsody™ Express Single-Cell Analysis 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.

Intended use

The BD Rhapsody™ 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 the BD Rhapsody™ Cartridge, which is a single-use consumable.

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

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

For more information on the purpose of the instruments, see the BD Rhapsody™ Express instrument overview ([page 10](#)) and the BD Rhapsody™ Scanner overview ([page 11](#)).

Restrictions

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

BD denies any responsibility for damage caused by the following:

- Any use of a BD Rhapsody™ Express instrument that does not comply with the procedures described in any guide used with the BD Rhapsody™ 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™ instrument warranty.

Disclaimer


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

Safety symbols

This topic describes the safety symbols used in this guide.

For safety and limitations, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner 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

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

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

Instrument technical support

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

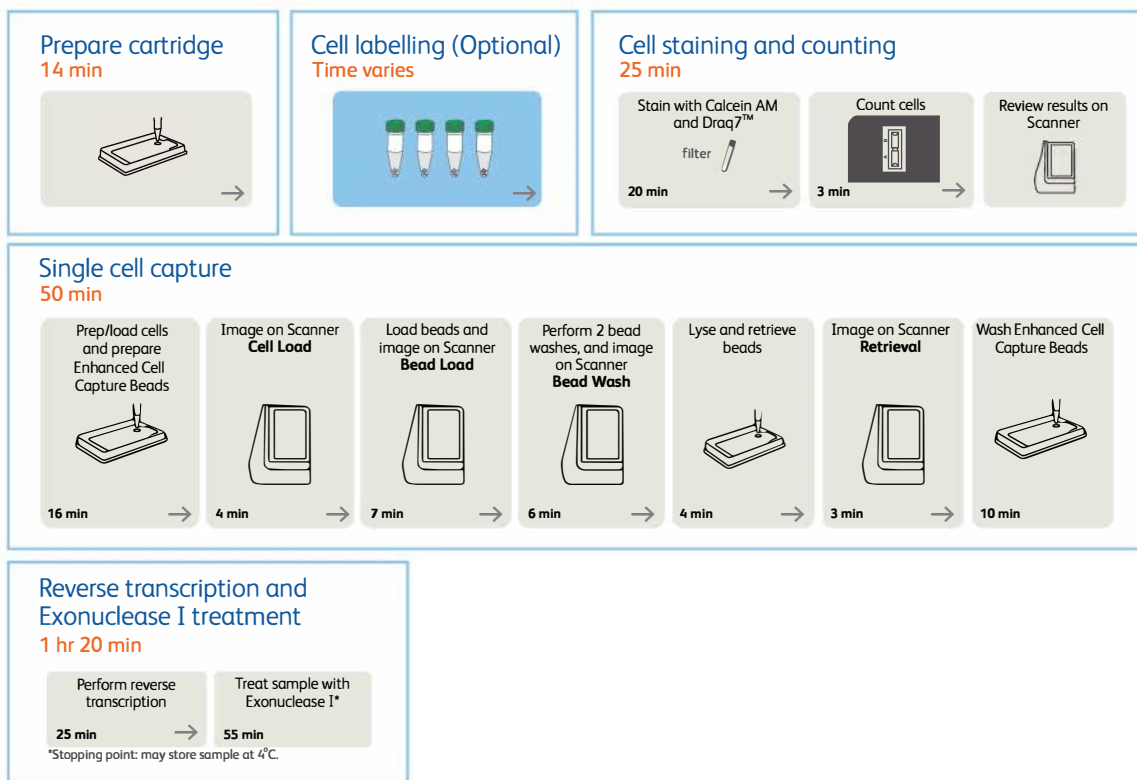
If technical assistance is required, contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com or 1.877.232.8995. You can contact technical support in Europe at help.biosciences@bd.com or +32.53.720.600.

Before contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number or lot number
- 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™ cartridge workflow



Note: When using two cartridges, see [Workflow with two BD Rhapsody™ Cartridges on page 59](#).

Site requirements

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

- Pre-amplification workspace
- Post-amplification workspace

For detailed site requirements and technical specifications, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner Site Preparation Guide* and [BD Rhapsody™ Scanner metrics on page 54](#).

For installation of the BD Rhapsody™ Express instrument and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ Single-Cell Analysis System 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.

Thermomixer settings

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

Thermal cycler setup

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

Pipette settings

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

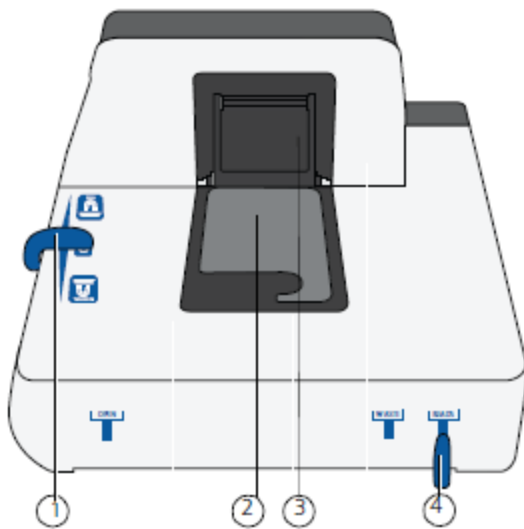
| Pipette | Mode |
|---------|-------------|
| P1200M | Prime/Treat |
| P1200M | Cell Load |
| P1200M | Bead Load |
| P1200M | Wash |
| P1200M | Lysis |
| P5000M | Retrieval |

BD Rhapsody™ Express instrument overview

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

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

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



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

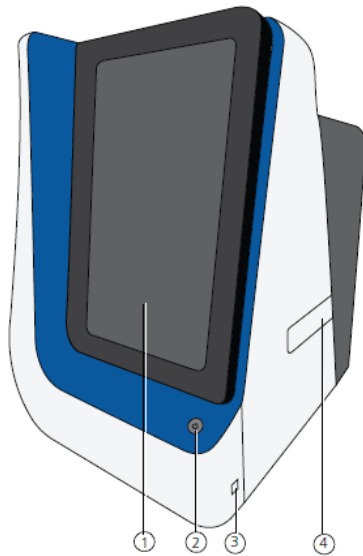
BD Rhapsody™ Scanner overview

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.

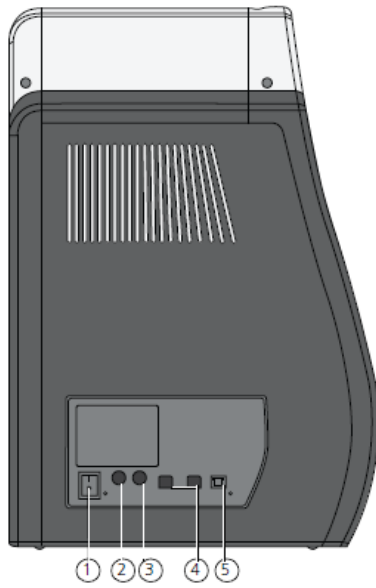
For safety and limitations of the scanner, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner Safety and Limitations Guide*.

See the *BD Rhapsody™ Single-Cell Analysis System 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. |

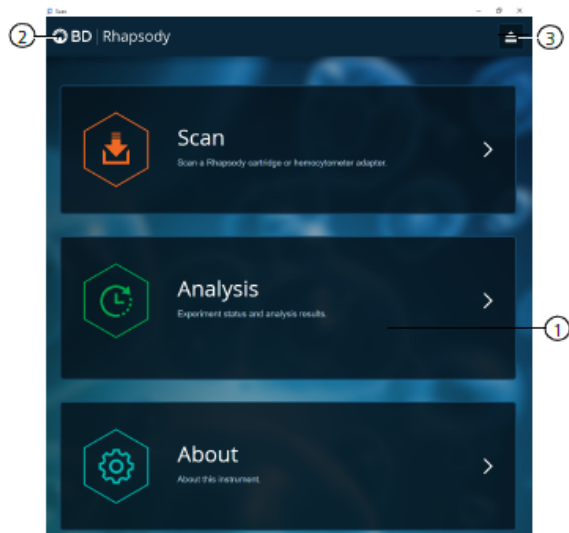


| No. | Rear component |
|-----|--------------------------------|
| 1 | Master power switch |
| 2 | Fuse holder |
| 3 | 24 VDC power input |
| 4 | USB 2.0 ports (2) ^a |
| 5 | Ethernet port |

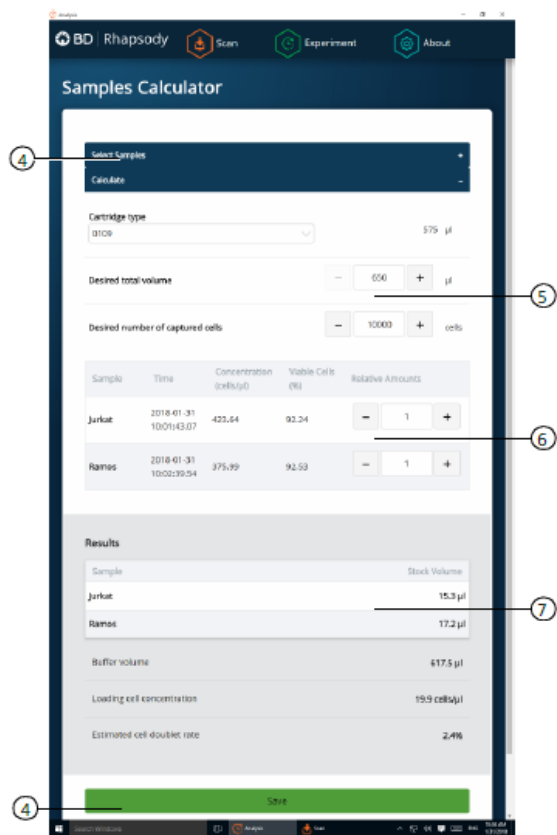
a. The USB 2.0 ports on the rear of the instrument are primarily used by BD Biosciences technical support.

Quick guide to the BD Rhapsody™ Scanner software

Navigation



| No. | Description |
|-----|---|
| 1 | Tap an application (app) to navigate between scan, analysis, and about screens. |
| 2 | Tap Rhapsody 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 Select Samples to display the window for selecting samples. Tap Calculate to calculate volumes for preparing cell suspensions. Tap Save on the Calculate tab to display saved calculations. |
| 5 | Enter the total volume and desired number of captured cells to prepare the cell suspension for loading into the BD Rhapsody™ Cartridge. |
| 6 | (Optional) Enter the relative amounts of samples that comprise the cell suspension. The default is 1:1. |
| 7 | Obtain the volumes of buffer and stock cells required to prepare the cell suspension for cartridge loading. |

BD Rhapsody™ Scanner software workflows

Scan application workflow

| Step | Screen |
|------|---|
| 1 | Main menu |
| 2 | Hemocytometer/cartridge placement on tray |
| 3 | Scan |
| 4 | Cartridge removal |

Analysis application workflow

| Step | Screen |
|------|-----------------------------------|
| 1 | Main menu |
| 2 | Experiment details |
| 3 | Prepare sample |
| 4 | Select sample |
| 5 | Calculate cell suspension volumes |
| 6 | Save calculation |
| 7 | Analysis metrics |

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™ Single-Cell Analysis System 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.
- Gently vortex solutions containing enzymes. Minimize vortex duration, and keep the vortex speed low. Do not vortex solutions containing BD Rhapsody™ Enhanced Cell Capture Beads.
- Gently pipet cells to avoid cell stress or death.
- Work in designated pre- or post-amplification workspaces according to the protocol.
- Prepare reagent mixes in pre-amplification workspaces, and conduct amplification in post-amplification workspaces.
- Wear suitable protective clothing, eyewear, and gloves.

RNase-free technique

Prevent the introduction of exogenous RNases into samples during processing:

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

Sterility

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

BD Rhapsody™ Enhanced Cell Capture Beads

- Always keep BD Rhapsody™ Enhanced Cell Capture Beads on ice when not in use.
Note: Do not freeze BD Rhapsody™ Enhanced Cell Capture Beads.
- For maximum recovery, do not vortex samples containing BD Rhapsody™ Enhanced Cell Capture Beads.
- Gently mix suspensions with BD Rhapsody™ Enhanced Cell Capture Beads by pipette only.
- Use low-retention tips and LoBind tubes when handling BD Rhapsody™ Enhanced Cell Capture Beads.

BD Rhapsody™ Express instrument

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

BD Rhapsody™ cartridge

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

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™ Cartridge Kit
- BD Rhapsody™ cDNA Kit

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

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

Required reagents

Required reagents

| Material | Supplier | Catalog no. |
|---|--------------------------|-------------|
| BD Rhapsody™ Enhanced Cartridge Reagent Kit | BD Biosciences | 664887 |
| BD Rhapsody™ Cartridge Kit | BD Biosciences | 633733 |
| BD Rhapsody™ cDNA Kit | BD Biosciences | 633773 |
| Absolute ethyl alcohol, molecule biology grade | Major supplier | – |
| Nuclease-free water | Major supplier | – |
| Calcein AM ^a | Thermo Fisher Scientific | C1430 |
| DRAQ7™ ^a | BD Pharmingen™ | 564904 |
| Dimethyl sulfoxide (DMSO) | Major supplier | – |
| 70% ethyl alcohol or 70% isopropyl alcohol ^b | – | – |

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™ Express instrument and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ Single-Cell Analysis System 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™ P1200M pipette | Thermo Fisher Scientific | F171803G |
| Gilson™ PIPETMAN™ Tipack™ Filter Tips, 500-5000 µL for BD Rhapsody™ P5000M pipette | Thermo Fisher Scientific | F161370G |
| Falcon® Tube with Cell Strainer Cap | Corning | 352235 |
| Falcon tubes, 5-mL Round Bottom Polystyrene Test Tube ^a | Corning | 352054 |
| DNA LoBind® Tubes, 1.5-mL | Eppendorf | 30108051 |
| DNA LoBind® Tubes, 5.0-mL ^b | Eppendorf | 30108310 |
| Low-retention, filtered pipette tips (10 µL, 200 µL, 1000 µL) | Major supplier | – |
| INCYTO™ disposable hemocytometer | INCYTO | DHC-N01-5 |
| 10 mL sterile serological pipettes | Major supplier | – |
| Premoistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol | Major supplier | – |
| Lint-free wipes | Major supplier | – |
| <p>a. Required for sample multiplexing and/or antibody-oligonucleotide labeling.</p> <p>b. These are the Bead Retrieval Tubes to be used with the BD Rhapsody™ Express instrument.</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 ^a | BD Biosciences | 633701 |
| BD Rhapsody™ Express Instrument ^a | BD Biosciences | 633702 |
| Hemocytometer Adapter ^a | BD Biosciences | 633703 |
| BD Rhapsody™ P1200M pipette ^a | BD Biosciences | 633704 |
| BD Rhapsody™ P5000M pipette ^a | BD Biosciences | 633705 |
| Large magnetic separation stand | V&P Scientific, Inc. | VP 772FB-1 |
| Clear acrylic cylinder adapter for 15-mL tube adapter ^b | V&P Scientific, Inc. | VP 772FB-1A |
| Microcentrifuge for 1.5–2.0-mL tubes ^c | Major supplier | – |
| Microcentrifuge for 0.2-mL tubes | Major supplier | – |
| Centrifuge and rotor with adapters for 5-mL Falcon tubes and 15-mL tubes | Major supplier | – |
| Pipet-Aid | Major supplier | – |
| Eppendorf ThermoMixer® C | Eppendorf | 5382000023 |
| SmartBlock™ Thermoblock 1.5-mL | Eppendorf | 5360000038 |
| Water bath OR incubator at 37 °C | Major supplier | – |
| Pipettes (P10, P20, P200, P1000) ^c | Major supplier | – |
| Vortexer ^c | Major supplier | – |
| Digital timer ^c | Major supplier | – |
| 6-Tube Magnetic Separation Rack for 1.5-mL tubes ^c | New England Biolabs | S1506S |
| Low-profile magnet separation stand for 0.2-mL, 8-strip tubes | V&P Scientific, Inc. Or, Clontech | VP 772F4-1 635011 |

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

Suggested equipment

Suggested equipment

| Equipment | Supplier | Catalog no. |
|--|----------------|-------------|
| Logitech® Wireless Combo (keyboard and mouse). | Major supplier | MK270 |
| Barcode reader with keyboard wedge, USB 2.0 OR 3.0 compatible. ^a | Major supplier | – |
| Phase-contrast microscope. | Major supplier | – |
| <p>a. The external barcode reader is optional. The scanner has an internal barcode reader. To purchase an external barcode reader, we recommend the USB Automatic Barcode Scanner Scanning Barcode Reader with Hands-Free Adjustable Stand (Brainydeal) or equivalent.</p> | | |

4. Preparing the BD Rhapsody™ Cartridge

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

Priming the BD Rhapsody™ Cartridge

Before you begin

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

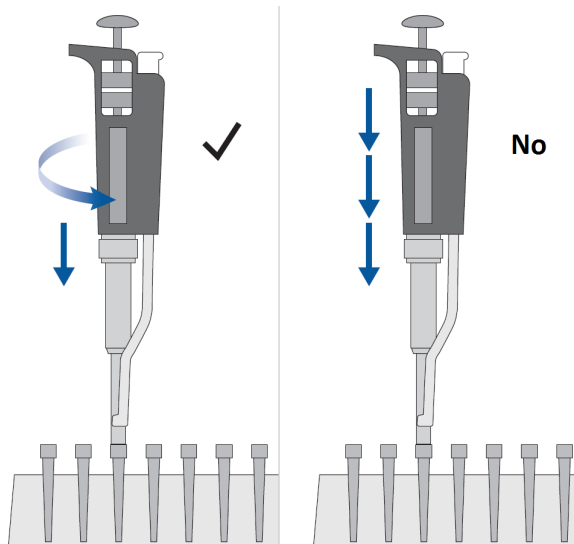
Visually inspect the Lysis Buffer for any precipitation. If precipitation is present, incubate the Lysis Buffer at room temperature (15–25 °C) for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, place the Lysis Buffer on ice.

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

- Equilibrate these reagents at room temperature (15–25 °C) within 30 minutes before use:
 - Cartridge Wash Buffer 1
 - Cartridge Wash Buffer 2
- Place these reagents on ice:
 - Sample Buffer
 - 1 M DTT
 - Lysis Buffer
 - DRAQ7 (protected from light)
- 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.
- Review pipette settings and operation. See [Pipette settings on page 9](#).
- For the use of two cartridges, see [Workflow with two BD Rhapsody™ Cartridges on page 59](#).

Procedure

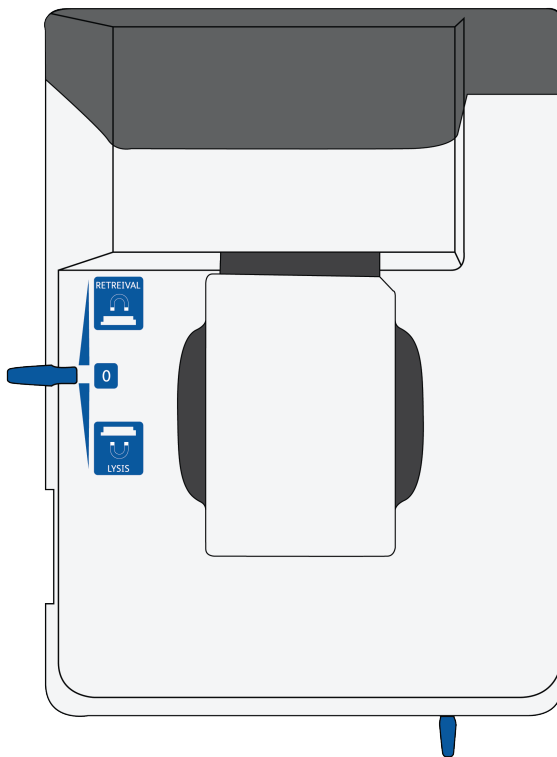
To ensure an air-tight seal with the BD Rhapsody™ P1200M or P5000M pipette, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft:



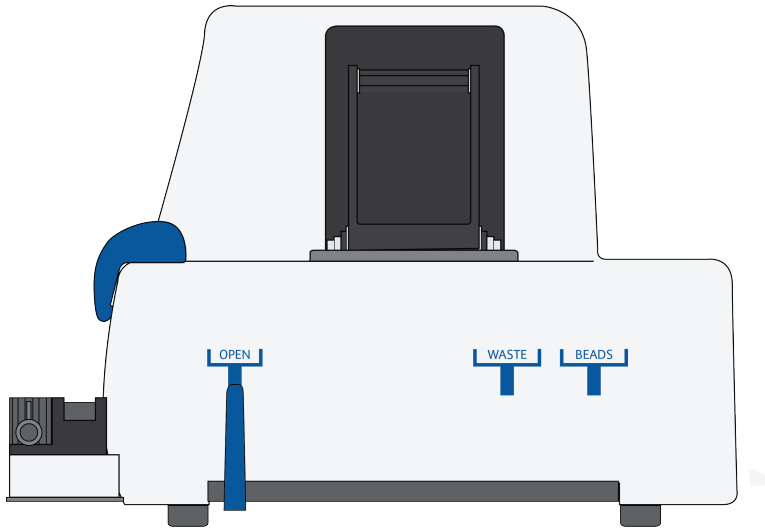
Avoid introducing bubbles while pipetting into the BD Rhapsody™ Cartridge.

Note: Change pipette tips before every pipetting step.

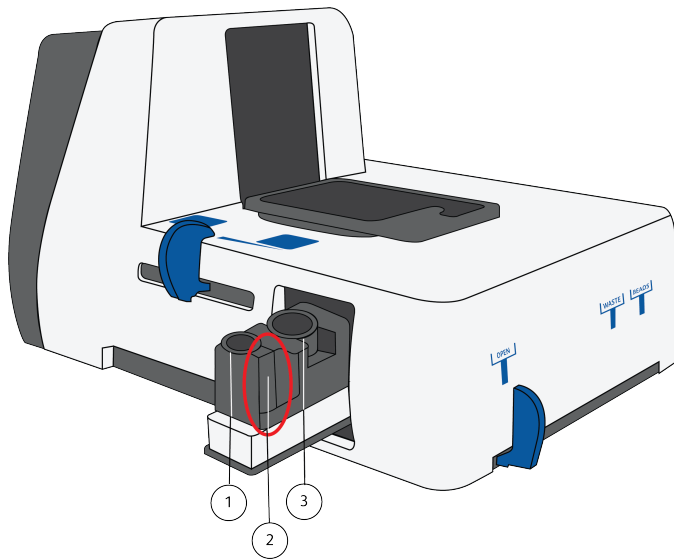
1. Move the left slider to the middle (0) position on the BD Rhapsody™ Express instrument. The Retrieval (top) magnet and Lysis (bottom) magnets are away from the cartridge tray:



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

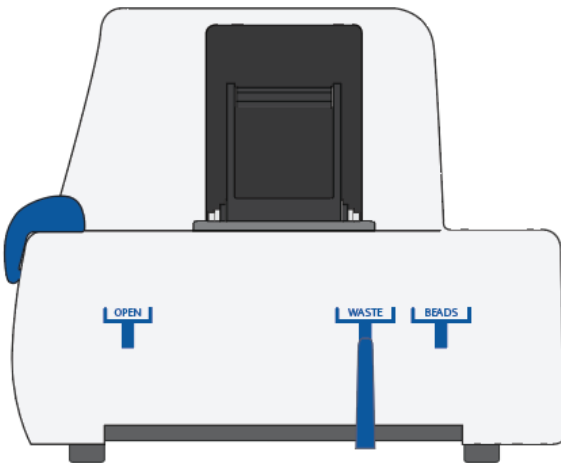


3. Remove the cap of a Waste Collection Container, and insert both the container and a new 5-mL LoBind tube for bead retrieval into the appropriate slots in the drawer. Secure the cap of the 5-mL LoBind tube to the holder:



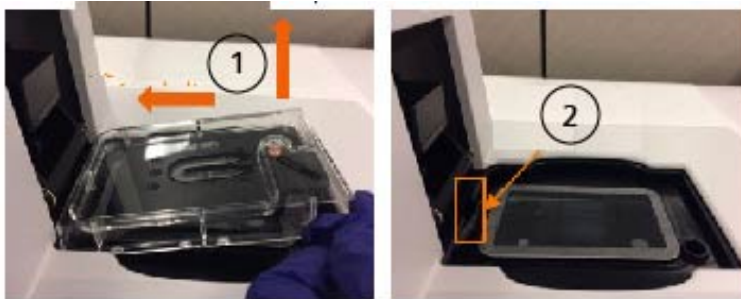
| No. | Component |
|-----|-------------------------------------|
| 1 | 5-mL LoBind tube for bead retrieval |
| 2 | Secure cap of 5-mL LoBind tube here |
| 3 | Waste Collection Container |

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



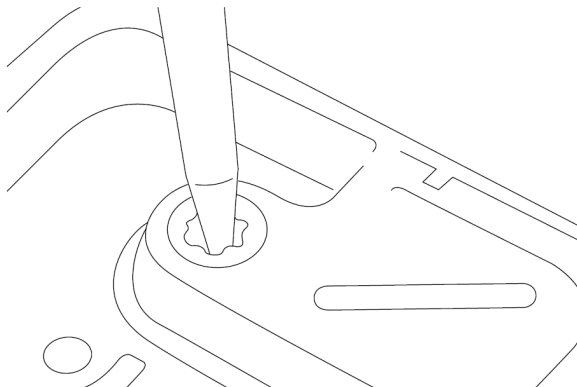
5. Push the cartridge into the far end of the tray to match the cartridge and tray notches. Lay the cartridge flat, and release it. Ensure that the cartridge is flat in the tray and the barcode faces out.

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



| No. | Component |
|-----|---------------|
| 1 | Push and lift |
| 2 | Metal clip |

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



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

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

Treating the surface of the cartridge

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

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

6. Prepare a single-cell suspension.

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

Best practices

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

- 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 μL).
- Cell counting the BD Rhapsody™ Scanner is most accurate when the cell concentration is in the range of ~200–800 cells/ μL . If the cell concentration is greater than 1,000 cells/ μL , dilute the cell suspension in cold Sample Buffer to ~200–800 cells/ μL .
- Do not rely on FACS-based counts, because cell concentration might be overestimated by this method. Always re-count 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. Refer to the *Preparing Single-Cell Suspensions Protocol*.
- Thaw Calcein AM. Once at room temperature (15–25 °C), resuspend Calcein AM in 503.0 μL DMSO for a final stock concentration of 2 mM. Follow manufacturer's instructions, and protect from light.

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 μL of residual supernatant. Add up to 620 μL 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 μL , 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 μL of 2 mM Calcein AM and 3.1 μL of 0.3 mM DRAQ7™ to 620 μL 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. We recommend filtering the final sample before loading cells into the cartridge.

6. Proceed immediately to [Loading cells into the Hemocytometer Adapter on page 28](#).

Loading cells into the Hemocytometer Adapter

Before you begin

Stain cells with viability markers. See [Staining cells with viability markers on page 27](#).

Note: Count cells immediately.

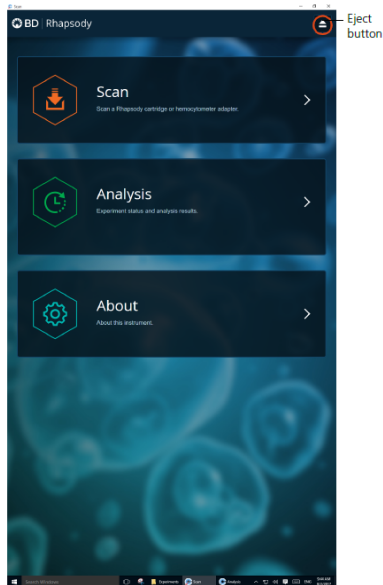
Loading cells

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

1. Gently mix cells well by pipette, and then gently pipet 10 μL 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. Wait for between 1 to 5 minutes after loading.
4. Count the cells in the BD Rhapsody™ Scanner.

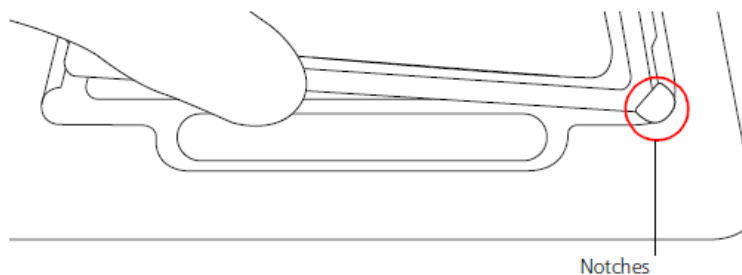
Counting cells with the BD Rhapsody™ Scanner

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.
 - To troubleshoot scanner software error messages, see [BD Rhapsody™ Scanner software messages on page 51](#).
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.



Note:

- The scanner displays an alert if the adapter is in the wrong orientation.
 - You can manually enter the barcode in the application.
4. Tap **Continue**. The tray retracts, the door closes, and the scanner displays the hemocytometer setup screen.
 5. Select the protocol from the drop-down menu. Enter the experiment name, sample name, protocol, and user. (If an experiment name was entered previously, select it from the drop-down menu.)

Note: Do not use commas in the experiment name, sample name, or user.

For example:

The screenshot shows a configuration screen with the following fields:

- Experiment: 20180131_SL_XH_Stage-Shaking-Run3
- Sample: Jurkat
- Protocol: Hemocytometer
- User: SL
- Cartridge: 0107001033A

Below the configuration fields, there are two main sections:

- Side A:** Contains a blue button labeled "Start Side A Scan".
- Side B:** A dark blue section, currently inactive.

Note: Enter an experiment name that is appropriate for both the hemocytometer and cartridge scans. If necessary, tap the touch keyboard icon in the bottom right on the screen to enter information. There are no character or length limits.

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.

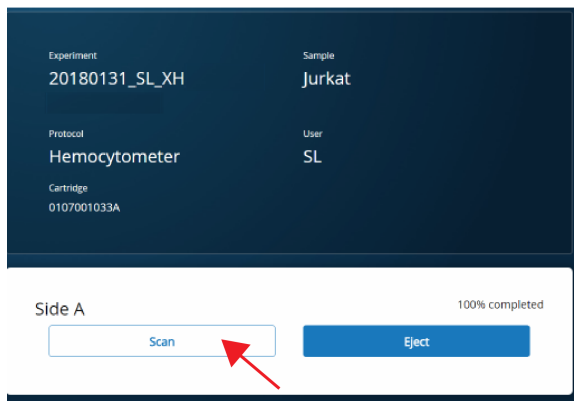
Note: To stop the scan, tap **Stop**, and then tap **Stop** again. To continue with the scan, tap **Continue**.

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

The screenshot shows the completion screen with the following elements:

- A green notification bar at the top: "Protocol step completed" with an "OK" button (indicated by a red arrow).
- Configuration information:
 - Experiment: 20180131_SL_XH
 - Sample: Jurkat
 - Protocol: Hemocytometer
 - User: SL
 - Cartridge: 0107001033A
- Side A section:
 - Status: 100% completed
 - Buttons: "Scan" (disabled) and "Eject" (active).

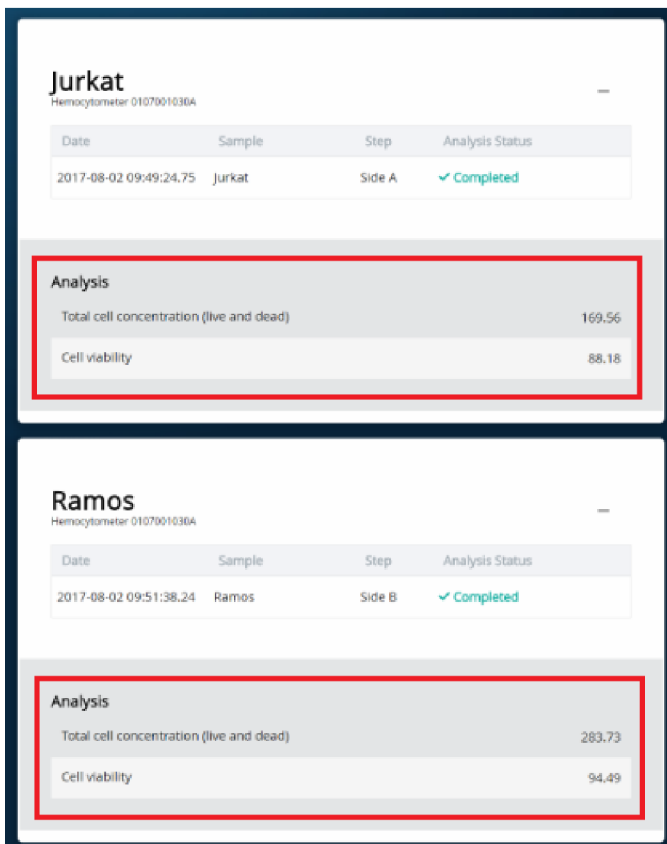
8. Scan the other side of the hemocytometer, or eject it:
 - Scan the other side: Tap **Scan**, enter a new sample name, and then repeat **steps 6–7**. The hemocytometer remains in the scanner and saves time waiting for the tray to eject. For example:



- 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 48](#).

12. Proceed as follows:
 - If the cell concentration is $\leq 1,000$ cells/ μL , proceed to **step 13**.
 - If the cell concentration is $> 1,000$ cells/ μL , dilute the cell suspension in cold Sample Buffer to ~ 200 – 800 cells/ μL . 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 the BD Rhapsody™ Cartridge

Best practices

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

Before you begin

- Prime and treat the BD Rhapsody™ Cartridge. See [Preparing the BD Rhapsody™ Cartridge on page 22](#).
- Thaw reagents (not enzymes) in the BD Rhapsody™ cDNA Kit at room temperature (15–25 °C), and then place them on ice. Keep enzymes at -25 °C to -15 °C.
- Prepare a single-cell suspension for cartridge loading.
- Place these reagents on ice:
 - Sample Buffer
 - 1 M DTT
 - Lysis Buffer
 - BD Rhapsody™ Enhanced Cell Capture Beads

Loading cells into the cartridge

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

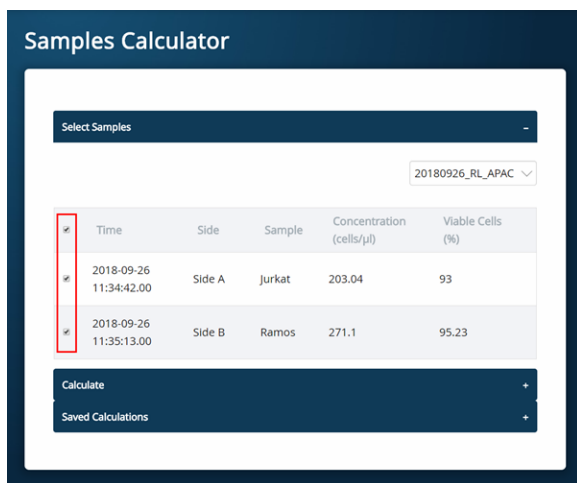
1. Determine the desired number of cells to capture in the BD Rhapsody™ Cartridge. The following table lists the estimated multiplet rate based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads:

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

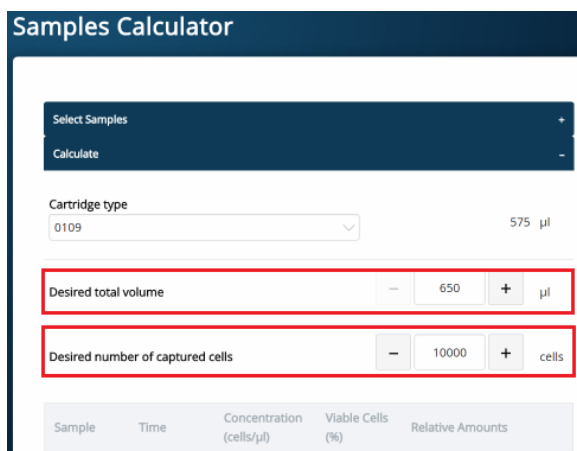
| Number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads (target) ^{a,b} | Estimated multiplet rate (%) |
|--|------------------------------|
| 100 | 0.0 |
| 500 | 0.1 |
| 1,000 | 0.2 |
| 2,000 | 0.5 |
| 3,000 | 0.7 |
| 4,000 | 1.0 |
| 5,000 | 1.2 |
| 6,000 | 1.4 |
| 7,000 | 1.7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

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 stock cell and volume of cold Sample Buffer to prepare a cell suspension of 650 µL for loading into a BD Rhapsody™ 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 650 μ L 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:

Desired number of captured cells cells

| Sample | Time | Concentration (cells/ μ l) | Viable Cells (%) | Relative Amounts |
|--------|---------------------------|--------------------------------|------------------|--|
| Jurkat | 2018-01-31 10:01:43.07 | 423.64 | 92.24 | <input type="button" value="-"/> <input type="text" value="1"/> <input type="button" value="+"/> |
| Ramos | 2018-01-31 10:02:39.54 | 375.99 | 92.53 | <input type="button" value="-"/> <input type="text" value="1"/> <input type="button" value="+"/> |

Results

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

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

Results

| Sample | Stock Volume |
|-----------------------------|---------------------|
| Jurkat | 15.3 μ l |
| Ramos | 17.2 μ l |
| Buffer volume | 617.5 μ l |
| Loading cell concentration | 19.9 cells/ μ l |
| Estimated cell doublet rate | 2.4% |

Save

- k. (Optional) 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.

Samples Calculator

Select Samples

Calculate

Saved Calculations

Cartridge type 0109 575 μ l Buffer volume 617.5 μ l

Desired total volume 650 μ l Loading cell concentration 19.9 cells/ μ l

Desired number of captured cells 10000 cells Estimated cell doublet rate 2.4 %

| Sample | Operator | Scan Time | Concentration (cells/ μ l) | Viable Cells (%) | Relative Amounts | Stock Volume |
|--------|----------|---------------------------|--------------------------------|------------------|------------------|--------------|
| Jurkat | | 2018-01-31 10:01:43.07 | 423.6 | 92.24 | 1 | 15.3 |
| Ramos | | 2018-01-31 10:02:39.54 | 376 | 92.53 | 1 | 17.2 |

4. Prepare the cell suspension.

| Stock cell volume or cell number | Cell suspension preparation |
|--|---|
| Calculated Stock Volume is <610 µL. | In a new 1.5-mL LoBind tube, prepare the cell suspension in cold Sample Buffer according to the volumes obtained in step 3 . |
| <ul style="list-style-type: none"> Calculated Stock Volume ≥610 µL, or Buffer volume is negative, or Cell concentration too low warning is displayed. | Use 610 µL of the cold cell suspension for cartridge cell loading. ^a |
| <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 610 µL. For example, if the Samples Calculator specifies mixing 700 µL of Jurkat cells and 30 µL of Ramos cells, mix 580 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.

- If the samples were not filtered before counting cells, pass the final dilution of the prepared single sample or pooled sample through a Falcon® tube with Cell Strainer Cap before loading the sample into the cartridge.
- Load the cartridge on the tray with 700 µL of air using the BD Rhapsody™ P1200M pipette in **Prime/Treat** mode.
- Change the mode of the BD Rhapsody™ P1200M pipette to **Cell Load**.
- With a manual pipette, gently pipet the cell suspension up and down to mix.
- On the BD Rhapsody™ P1200M pipette, press the pipette button once to aspirate 40 µL of air, immerse the pipette tip in cell suspension, and then press the button again to aspirate 575 µL of cold cell suspension.
- Insert the tip of the pipette perpendicular to the port, seal the pipette tip against the gasket, and then press the button a third time to dispense 615 µL of air and cells.

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

- Incubate the cells by one of these methods:
 - On the BD Rhapsody™ Express instrument: Leave the cartridge with loaded cells on the tray at room temperature (15–25 °C) for 15 minutes. During incubation on the laboratory bench, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See [Preparing BD Rhapsody™ Enhanced Cell Capture Beads on page 39](#). After preparing the BD Rhapsody™ Enhanced Cell Capture Beads, proceed to [Imaging cells in a cartridge on page 36](#), or
 - In the scanner: Proceed immediately to [Imaging cells in a cartridge on page 36](#) to install the cartridge and enter a 15 minute run delay.

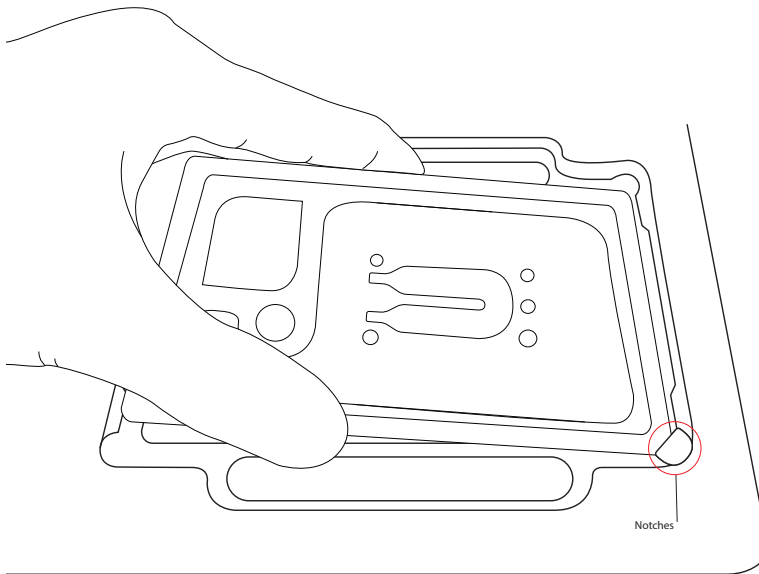
Imaging cells in a cartridge

- 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.

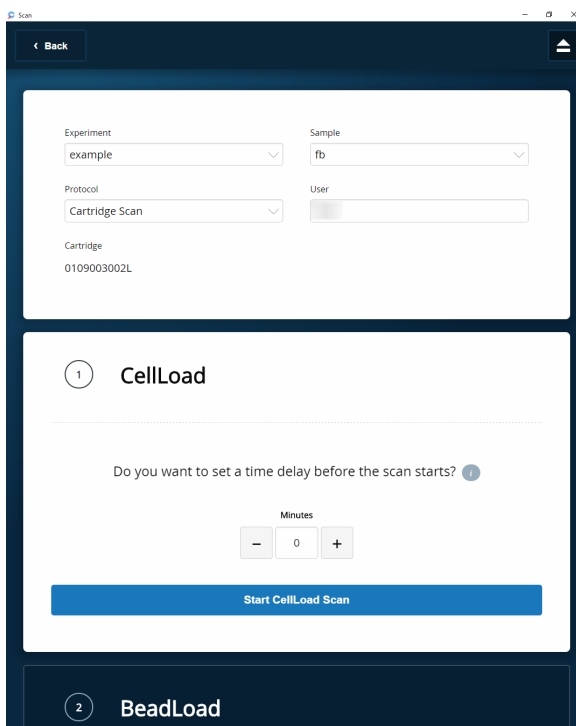
- Push the cartridge into the far end of the Express instrument tray to match the cartridge and tray notches. Lay the cartridge flat, and release it. Ensure that the cartridge is flat in the tray and the barcode faces out.
- If necessary, wipe condensation from the top surface of the cartridge with a lint-free wiper 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.
5. Tap **Continue**. The tray retracts, the door closes, and the scanner displays the experiment setup screen.
 6. Select from the drop-down menu or enter the experiment name, sample name, protocol, and user.
 7. Tap **Cell Load**.



8. Proceed according to incubation location:

- Express instrument (15 minute incubation completed):
Proceed to **step 9**.
- Incubation in scanner: Enter a 15 minute time delay to delay the start of the scan, 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 39](#). 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 15 minute countdown time displays in the Cell Load window. The scan proceeds after the countdown completes. The scan takes an additional ~4 minutes to complete.

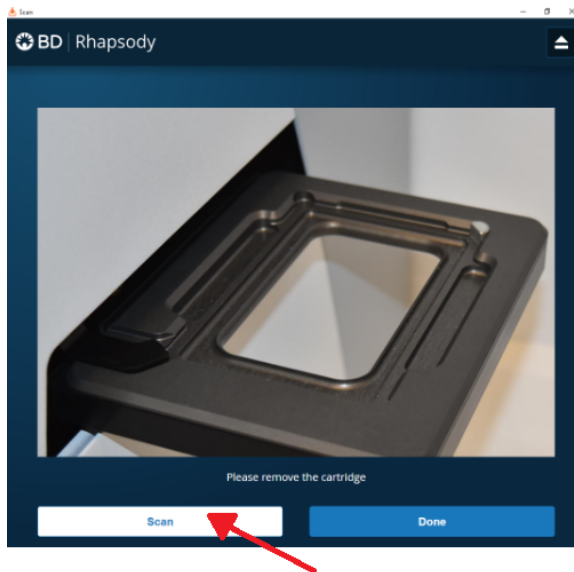
Note: To stop the scan, tap **Stop**, and then tap **Stop** again. You can then scan the cartridge with a different step or rerun the scan. To continue with the current scan, tap **Continue**.

10. After the scan is complete, 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.

11. Scan another cartridge, or retract the tray:

- Scan another cartridge: Tap **Scan**, and proceed to **step 4** to place a new cartridge on the tray for scanning.



- Retract the tray: Tap **Done**.
12. 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.

Ctrl-85pct

Cartridge 0109033258A

| Scan Date | Sample | Step | Analysis Status |
|------------------------|------------|-----------|-----------------|
| 2018-01-31 10:26:00.49 | Ctrl-85pct | Cell Load | ✓ Completed |

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

Preparing BD Rhapsody™ Enhanced Cell Capture Beads

Before you begin

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

Preparing BD Rhapsody™ Enhanced Cell Capture Beads

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

1. Place the tube with BD Rhapsody™ Enhanced Cell Capture Beads on the 1.5-mL tube magnet for 1 minute.
2. Carefully remove and appropriately discard the storage buffer without disturbing the beads and while leaving the tube on the magnet.
3. Remove the tube from the magnet, and then pipet 750 μ 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. 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 40](#).

Loading BD Rhapsody™ Enhanced Cell Capture Beads and imaging

1. Return the cartridge to the tray of the BD Rhapsody™ Express instrument.
2. Change the mode of the BD Rhapsody™ P1200M pipette to **Prime/Treat**.
3. Load the cartridge with 700 µL of air using the BD Rhapsody™ P1200M pipette in **Prime/Treat** mode.
4. Change the mode of the BD Rhapsody™ P1200M pipette to **Bead Load**.
5. Use a P1000 standard pipette to gently pipet the BD Rhapsody™ Enhanced Cell Capture Beads in cold Sample Buffer up and down to mix, and, using the BD Rhapsody™ P1200M pipette in **Bead Load** mode, immediately load the cartridge with 630 µL of beads.
6. Let the beads settle in the cartridge on the tray at room temperature (15–25 °C) for 3 minutes.
7. Image the cartridge with the scanner (Bead Load step). For detailed instructions, see [Imaging cells in a cartridge on page 36](#).
8. Return the cartridge to the tray of the BD Rhapsody™ Express instrument.
9. Change the mode of the BD Rhapsody™ P1200M pipette to **Wash**.
Note: In **Wash** mode, press the button once to aspirate 720 µL of air or reagent. After aspiration, insert the tip into the cartridge, and then press the button once to dispense 700 µL of air or liquid. After removing the pipette tip from the cartridge inlet, press the button once to dispense the remaining 20 µL of air or liquid before ejecting the pipette tip.
10. Load the cartridge with 700 µL of air using the BD Rhapsody™ P1200M pipette in **Wash** mode.
11. Load the cartridge with 700 µL of cold Sample Buffer using the BD Rhapsody™ P1200M pipette in **Wash** mode.
12. Repeat **steps 10–11** once for a total of two washes.
13. Image the cartridge with the scanner (**Bead Wash** step). For detailed instructions, see [Imaging cells in a cartridge on page 36](#).

Lysing cells and retrieving BD Rhapsody™ Enhanced Cell Capture Beads

Lysing the cells

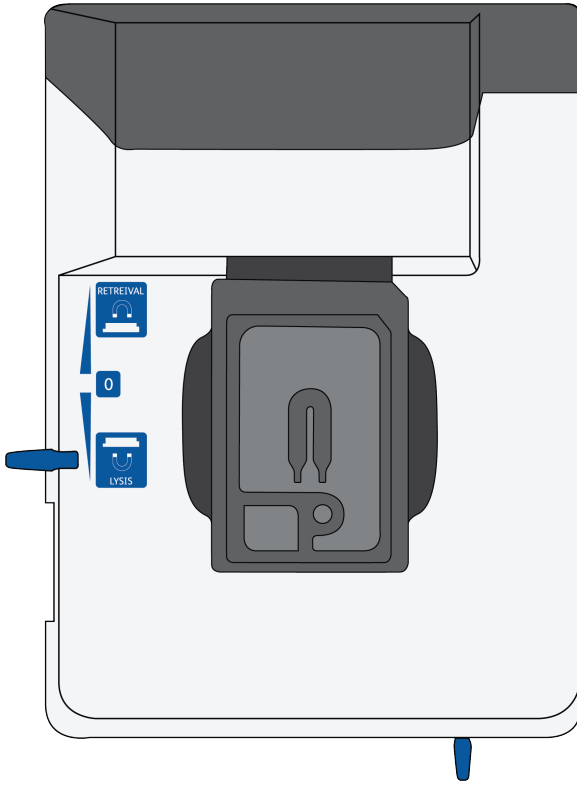
Avoid bubbles.

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

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

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

2. Briefly vortex the lysis mix, and place it on ice.
3. Return the cartridge to the tray of the BD Rhapsody™ Express instrument.
3. Move the left slider to **LYSIS**. The (bottom) magnet is in the up position and is in contact with the cartridge.

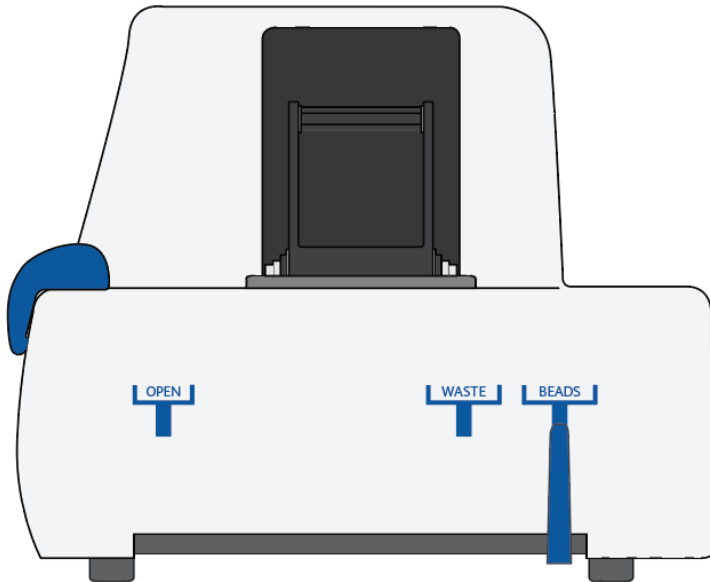


4. Change the mode on the BD Rhapsody™ P1200M pipette to **Lysis**.
5. Load the cartridge with 550 μ L of Lysis Buffer with DTT using the BD Rhapsody™ P1200M pipette in **Lysis** mode.
6. Leave the cartridge at room temperature (15–25 °C) on the tray for 2 minutes.

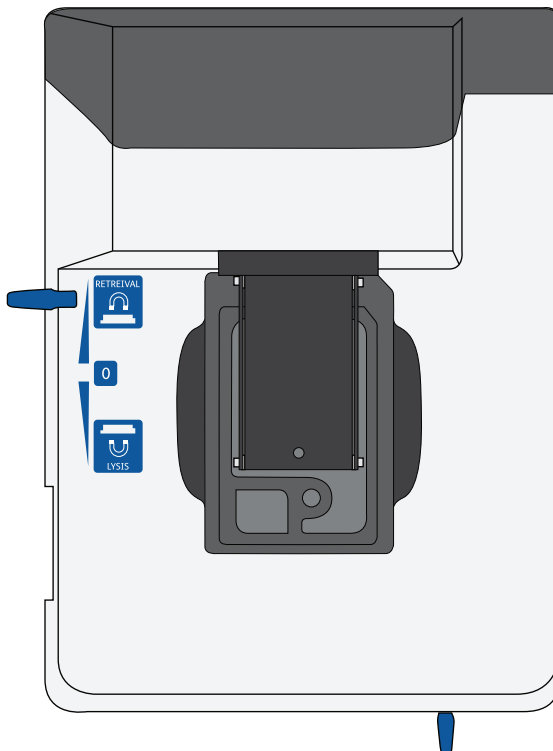
Note: Maintain recommended lysis time for best performance.

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

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



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



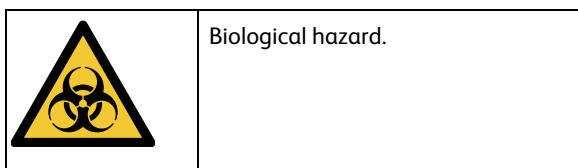
5. Leave the Retrieval magnet in the down position for 30 seconds.
6. Use the BD Rhapsody™ P5000M pipette to aspirate 5,000 µL of Lysis Buffer with DTT.
7. Press down firmly on the BD Rhapsody™ P5000M pipette to seal the pipette tip against the gasket of the cartridge to avoid leaks.
8. Move the left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

The BD Rhapsody™ Enhanced Cell Capture Beads are collected in the 5-mL LoBind tube.

9. Remove the pipette tip from the inlet gasket of the cartridge before pressing the dial button once to purge the tip. Discard the pipette tip.
10. Move the front slider to **OPEN**, and then remove and cap the 5-mL LoBind tube.
11. Uncap the 5-mL LoBind tube, and place it on the large magnetic separation stand fitted with the 15-mL tube adapter for 1 minute.

Note: During the 1 minute incubation, proceed to imaging the cartridge in **step 12**.

12. Image the cartridge with the scanner (**Retrieval** step). For detailed instructions, see [Imaging cells in a cartridge on page 36](#).
13. Proceed immediately to [Performing reverse transcription on the BD Rhapsody™ Enhanced Cell Capture Beads on page 44](#) to process the beads and begin reverse transcription.
14. Appropriately dispose of the BD Rhapsody™ Cartridge according to biosafety level (BSL).



- 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 61](#).

15. Appropriately dispose of the waste in the Waste Collection Container.
16. Appropriately dispose of the Lysis Buffer with DTT.
17. Wipe the Express instrument with 10% (v/v) bleach or 70% (v/v) ethyl alcohol. See the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide*.

Washing the BD Rhapsody™ Enhanced Cell Capture Beads

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

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

1. After the 1 minute incubation on the large magnet [see [Lysing cells and retrieving BD Rhapsody™ Enhanced Cell Capture Beads on page 40](#)] and while leaving the 5-mL LoBind® tube on the large magnet, use a pipette to carefully remove all but ~1 mL of supernatant without disturbing the beads.
2. Remove the tube from the large magnet, resuspend the ~1 mL beads by gently pipetting the suspension up and down, and then transfer the bead suspension to a new 1.5-mL LoBind® tube.

- If the beads remain in the 5-mL LoBind tube, pipet an additional 0.5 mL of Lysis Buffer with DTT into the 5 mL tube, rinse the 5 mL tube, and transfer the suspension to the 1.5-mL LoBind® tube of beads.
- Place the tube on the 1.5-mL tube magnet for up to and including 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.

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

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

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

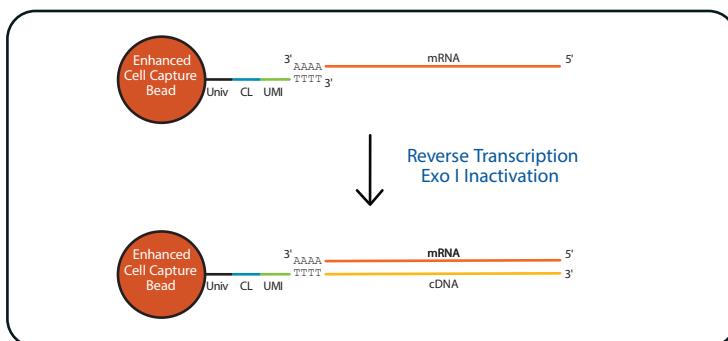
STOP!

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

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

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

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



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

Best practices

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

Before you begin

- Obtain the 5-mL LoBind[®] tube of retrieved beads. See [Retrieving the BD Rhapsody™ Enhanced Cell Capture Beads from the cartridge on page 42](#).
- Ensure that the SmartBlock™ Thermoblock 1.5-mL or equivalent is installed on the thermomixer and is set to 37 °C for 20 minutes.

Performing reverse transcription

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

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

Note: Prepare the cDNA mix on ice.

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

cDNA mix

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

3. Gently vortex and centrifuge the mix, and then place it back on ice.
4. Place the tube of washed beads (see [Washing the BD Rhapsody™ Enhanced Cell Capture Beads on page 43](#)) on the 1.5-mL tube magnet for up to 2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
5. Use a low-retention tip to pipet 200 μL of the cDNA mix to resuspend the beads. Gently mix the suspension by pipette only. Do not vortex.

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

6. Transfer the bead suspension to a new 1.5-mL LoBind[®] tube.

7. Incubate the suspension on the thermomixer at 1,200 rpm and 37 °C for 20 minutes.

Note: Shaking is critical for this incubation.

Note: During reverse transcription incubation, view the image analysis to determine if the analysis metrics have passed. See [Reviewing the analysis metrics on page 56](#).

8. After incubation, place the tube on ice.

Treating the sample with Exonuclease I

Before you begin

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

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

Preparing the Exonuclease I mix

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

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

Note: Prepare the Exonuclease I mix on ice.

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

Exonuclease I mix

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

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

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

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

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

4. Immediately proceed to [Inactivating Exonuclease I on page 47](#).

Inactivating Exonuclease I

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

Do not exceed this inactivation temperature and incubation time.

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

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

6. Proceed to library preparation.

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™ Express instrument 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 scomix@bdscomix.bd.com . |

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 54](#).

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"> Confirm that the calculation of cell dilution is correct. Reduce the number of cells loaded in the cartridge. |
| | Improper counting with hemocytometer | <ul style="list-style-type: none"> Recount cells with the BD Rhapsody™ Scanner. Follow Processing cells with the BD Rhapsody™ Single-Cell Analysis system on page 27. |
| | Incorrect calculation of cell concentration | <ul style="list-style-type: none"> Use the BD Rhapsody™ Scanner for cell counting and analysis. Use the Analysis app Sample Calculator for preparing cell suspensions. |
| Number of cells captured lower than anticipated. | Incorrect calculation of cell concentration. | <ul style="list-style-type: none"> Use the BD Rhapsody™ Scanner for cell counting and analysis. Use the Analysis app Sample Calculator for preparing cell suspensions. |
| | Cell size larger than recommended range. | <ul style="list-style-type: none"> BD Rhapsody™ Enhanced Cell Capture Beads efficiency is reduced in the BD Rhapsody™ Scanner if the cell diameter is >20 µm. |
| | Insufficient incubation after cell loading. | <ul style="list-style-type: none"> Make sure incubation time after cell loading is 15 minutes and not less. |
| Bead loading density out of range. | Insufficient number of BD Rhapsody™ Enhanced Cell Capture Beads loaded. | <ul style="list-style-type: none"> Ensure that all of the beads are pelleted before removing storage buffer and are resuspended in Sample Buffer. Use the Bead Load mode on the pipette. Ensure the beads are well suspended before loading into the cartridge. |
| 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"> Ensure that the correct pipette mode is used for the step. |
| | Pipette step missed. | <ul style="list-style-type: none"> Review protocol, and repeat, if necessary. |

Cartridge loading troubleshooting (continued)

| Observation | Possible causes | Recommended solutions |
|--|--|--|
| Cell retention out of range, indicating cell loss during cartridge workflow. | Pipette mode incorrect. | <ul style="list-style-type: none"> Ensure that the correct pipette mode is used for the step. |
| | Cell viability low. | <ul style="list-style-type: none"> Use cells of high viability. |
| Bead retrieval efficiency out of range, indicating poor retrieval. | Retrieval (top) magnet not positioned properly. | <ul style="list-style-type: none"> Check magnet position. |
| | Pipette mode incorrect. | <ul style="list-style-type: none"> Ensure that the correct pipette mode is used for the step. |
| Image analysis does not start. | Software does not initiate analysis after a scan. | <ol style="list-style-type: none"> On the BD Rhapsody™ Scanner main menu, tap Analysis. Tap the experiment. Tap Analyze to start the analysis. You can analyze multiple scan steps at the same time. |
| Dropped the cartridge or hit it against object. | Various. | <ul style="list-style-type: none"> If the cartridge was dropped, we recommend using a new cartridge; otherwise, carefully review the imaging metrics before proceeding. If the cartridge was struck, proceed at your own risk, and carefully review the imaging metrics before proceeding. |
| Air bubble in cartridge. | Air bubble present in pipette tip while dispensing buffer. | <ul style="list-style-type: none"> Confirm that there is an air bubble in the cartridge by examining an image of the cartridge. Ensure that the pipette tip contains only buffer and no air bubble is trapped in the cartridge at the end of aspiration. |
| | Re-used pipette tip. | <ul style="list-style-type: none"> Use a new pipette tip at every pipetting step. |
| Image analysis fails. | Contact BD Biosciences technical support at scomix@bdscomix.bd.com . | |
| Installation or mechanical issues. | See the <i>BD Rhapsody™ Single-Cell Analysis System 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.

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"> 1. Remove obstruction, and retry retracting cartridge into the scanner. 2. Click Cancel. 3. Restart the scanner. |
| “Available disk space is extremely low.” | Low disk space. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Failed to initialize...” | Various. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Requested move not safe.” | Various | Contact BD tech support scomix@bdscomix.bd.com . |
| “Unknown cartridge barcode.” | Unsupported barcode scanned | Contact BD tech support scomix@bdscomix.bd.com . |
| “Failed to read file.” | Various | Contact BD tech support scomix@bdscomix.bd.com . |
| “Cartridge alignment feature not found.” | <ul style="list-style-type: none"> • Incorrect cartridge • Light failed | 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. | 1. Reinsert the cartridge, and rescan the cartridge. 2. If necessary, restart the scanner. |
| “Available disk space is running low.” | Limited disk space. | Contact BD tech support scomix@bdscomix.bd.com . |
| “The protocol was not found.” | Protocol missing. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Auto-exposure is not configured for image type. Using default value instead.” | Method not found. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Auto-exposure resulted in images darker than normal.” | <ul style="list-style-type: none"> • No cell in image tile. • Light failed. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Auto-exposure resulted in images brighter than normal.” | Various. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Auto-exposure adjustment unsuccessful. Using nearest value.” | Algorithm calculation. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Autofocus did not find perfect focus. Using nearest value.” | <ul style="list-style-type: none"> • Bubble in cartridge. • Calibration needs update. | Contact BD tech support scomix@bdscomix.bd.com . |
| “Attempted to use exposure time greater than maximum. Using the maximum instead.” | Light failed. | Contact BD tech support scomix@bdscomix.bd.com . |
| “No alignment marks found. Continuing without cartridge specific calibration.” | Cartridge misaligned. | Reinsert cartridge, and rescan. |
| “Not enough local alignment marks detected.” | <ul style="list-style-type: none"> • Bead is covering an alignment mark. • Bad image. | Contact BD tech support scomix@bdscomix.bd.com . |

Warning messages (continued)

| Warning message | Possible causes | Recommended solutions |
|--|---|--|
| "Not enough local fiducials detected." | <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> | Contact BD tech support scomix@bdscomix.bd.com . |

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™ 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. ^a | – |
| 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. ^b | – |
| 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. ^c | 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. ^c | – |

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. $BD\ Rhapsody^{TM}\ Enhanced\ Cell\ Capture\ Beads\ cell\ capture\ efficiency\ at\ Cell\ Load\ (\%) = \frac{\text{Number of viable cells captured in well at Cell Load}}{\text{Total number of viable cells imaged}} * 100.$</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 Processing cells with the BD Rhapsody™ Single-Cell Analysis system on page 27. The cell multiplet rate reported by the BD Rhapsody™ Scanner is determined by imaging. If the cell multiplet rate >> 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.

The Rhapsody Data folder contains ExperimentName_SampleName.csv, PrepareSampleResults.csv, and Cell Count.csv.

Reviewing the analysis metrics

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

For example:

Dispense1-1
Cartridge 0109034055A

| Scan Date | Sample | Step | Analysis Status |
|------------------------|-------------|-----------|-----------------|
| 2018-02-21 10:29:27.56 | Dispense1-1 | Cell Load | ✓ Completed |
| 2018-02-21 10:57:04.44 | Dispense1-1 | Bead Load | ✓ Completed |
| 2018-02-21 11:11:49.59 | Dispense1-1 | Bead Wash | ✓ Completed |
| 2018-02-21 11:26:42.94 | Dispense1-1 | Retrieval | ✓ Completed |

3. Review the analysis metrics. For PASS criteria, see [Image analysis metrics on page 54](#). For example:

| Analysis | |
|--|--------|
| Number of wells with viable cells at cell load | 9118 |
| Cell multiplet rate at cell load | 2.4 % |
| Number of wells with viable cells and a bead | 7999 |
| Cell multiplet rate | 2.0 % |
| 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 16](#).

- [\(1\) Analysis metrics on page 57](#)
- [\(2\) Cell count on page 58](#)
- [\(3\) Sample result on page 58](#)

(1) Analysis metrics

| | |
|---|----------------|
| Barcode | 0109003003A |
| Experiment | Dispense 1-1 |
| Sample | Dispense 1-1 |
| Scan System ID | R-1016 |
| Scan start date and time | 6/5/2019 10:20 |
| Operator | PC |
| Analysis Software Version | 1.3.0.12 |
| Cell load analysis last updated | 6/5/2019 11:10 |
| Total number of tiles | 26 |
| Cell load number of tiles processed | 26 |
| Number of wells with viable cells at cell load | 4001 |
| Number of viable cells captured in wells at cell load | 4052 |
| Cell multiplet rate at cell load | 1.30% |
| Bead wash analysis last updated | 6/5/2019 11:37 |
| Bead wash number of tiles processed | 26 |
| Number of wells with viable cells and a bead | 3665 |
| Number of viable cells captured in wells with a bead | 3710 |
| Cell multiplet rate | 1.20% |
| Bead loading efficiency | 94.70% |
| Bead loading acceptance | PASS |
| Excess bead rate | 0.00% |
| Excess bead acceptance | PASS |
| Cell retention rate | 96.90% |
| Cell retention acceptance | PASS |
| Bead retrieval analysis last updated | 6/5/2019 11:47 |
| Bead retrieval number of tiles processed | 26 |
| Bead retrieval efficiency | 95.90% |
| Bead retrieval acceptance | PASS |
| Bead retrieval efficiency relative to bead wash | 95.70% |
| Number of retrieved beads with 1+ viable cells | 3507 |

(2) Cell count

| | |
|--|-----------------------------------|
| Analysis software version | 1.3.0.12 |
| Experiment | 20190528_PC_CartridgeSWValidation |
| Sample | Jurkat |
| Barcode | 0107001031A |
| Protocol step | Side A |
| Scan System ID | RESOLVE-1017 |
| Operator | PC |
| Scan start date and time | 5/28/2019 10:59 |
| Analysis last updated | 6/5/2019 9:46 |
| Total cell concentration (live and dead) | 1645.89 |
| Cell viability | 94.98 |

| | |
|--|-----------------------------------|
| Analysis software version | 1.3.0.12 |
| Experiment | 20190528_PC_CartridgeSWValidation |
| Sample | Ramos |
| Barcode | 0107001031A |
| Protocol step | Side B |
| Scan System ID | RESOLVE-1017 |
| Operator | PC |
| Scan start date and time | 5/28/2019 11:00 |
| Analysis last updated | 6/5/2019 9:47 |
| Total cell concentration (live and dead) | 1697.5 |
| Cell viability | 94.65 |

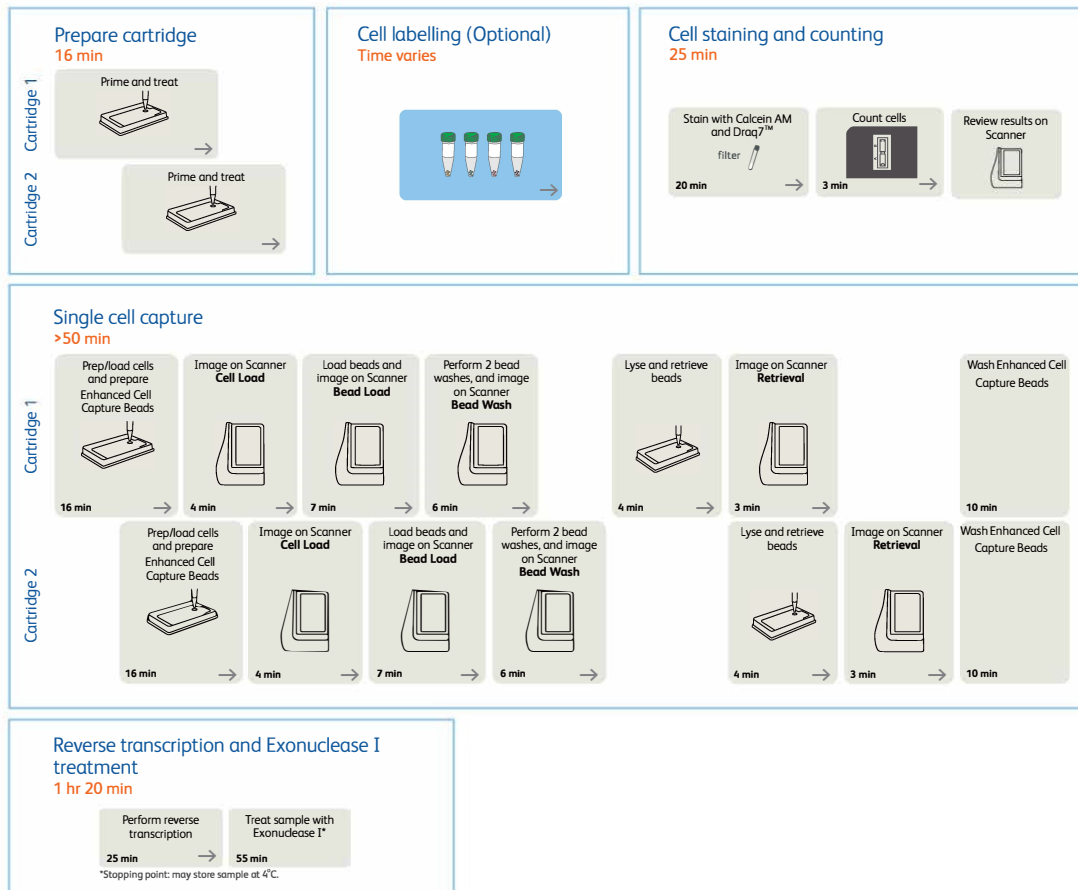
(3) Sample result

| | | | | | | |
|-------------------------------|-----------------------------------|--------------------|---------------|--------------|-----------------|-------------------|
| Experiment | 20190528_PC_CartridgeSWValidation | | | | | |
| Cartridge type | 109 | | | | | |
| Cartridge volume(uL) | 575 | | | | | |
| Total volume to prepare (uL) | 1500 | | | | | |
| Desired number of captured ce | 10000 | | | | | |
| Sample buffer volume (uL) | 1479.6 | | | | | |
| Loading cell concentration | 22.8 | | | | | |
| Cell doublet rate (%) | 2.4 | | | | | |
| Sample | Operator | Scan Date and Time | Concentration | Viable Cells | Relative Amount | Stock Volume (uL) |
| Jurkat | PC | 57:49.7 | 1668.4 | 95.14 | 1 | 10.2 |
| Ramos | PC | 58:38.2 | 1686.4 | 94.7 | 1 | 10.1 |

8. Workflow with two BD Rhapsody™ Cartridges

Workflow with two cartridges

Staggered boxes indicate staggering the start of like steps.



Best practices with a two-cartridge workflow

Reagent preparation

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

9. Updating the BD Rhapsody™ Scanner software

We recommend updating the scanner software by navigating the installation with keyboard and mouse. For recommended peripherals, see a BD Rhapsody™ user guide.

For updating BD Rhapsody™ Scanner software, see the *BD Rhapsody™ Scanner Software Installation Guide*.

10. Safety

General safety and limitations

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

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™ 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"> • 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 | <ul style="list-style-type: none"> • Frequency of Handling: every BD Rhapsody™ experiment. • Content: polymer (99%), polymer micro particles (<1%), Lysis Buffer (<1%). • Main Risk Constituent(s): Lysis Buffer. • Collect and dispose of all used BD Rhapsody™ Cartridges using proper precautions and according to local safety regulations. |

Physical safety

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

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

Instrument waste disposal

Contact BD Biosciences technical support at scomix@bdscomix.bd.com before moving the BD Rhapsody™ Scanner or disposing of the BD Rhapsody™ Express instrument or the BD Rhapsody™ Scanner. For more information, see [Instrument technical support on page 7](#).

Becton, Dickinson and Company

BD Biosciences

2350 Qume Drive

San Jose, California 95131 USA

bdbiosciences.com

scomix@bdscomix.bd.com