

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

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## History

Revision	Date	Change made
Doc ID: 214062 Rev. 1.0	7/2018	Initial release

Revision	Date	Change made
Doc ID: 214062 Rev. 2.0 23-21336-00	2/2019	Added information about the BD™ Mouse Immune Single-Cell Multiplexing Kit.
Doc ID: 214062 Rev. 3.0 23-21336-01	8/2019	<ul style="list-style-type: none"> <li>—Updated analysis metrics, cell count, and sample result for BD Rhapsody™ Scanner.</li> <li>—Updated error and warning messages for BD Rhapsody™ Scanner software.</li> <li>—Added reference to <i>BD Rhapsody™ Scanner Software Installation Guide (Part Number: 23-20709-01)</i> in Appendix C: Updating the BD Rhapsody™ Scanner software.</li> <li>—Updated BD Biosciences technical support Email address to <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a>.</li> <li>—Removed BD Rhapsody™ Training Cells Kit information from the Required kits and storage conditions section.</li> </ul>



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# 1

## Introduction

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- About this guide (page 8)
- Intended use (page 8)
- Safety symbols (page 10)
- Safety data sheets (page 12)
- Instrument technical support (page 13)

## About this guide

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### Introduction

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* (Doc ID: 214063).

Genomics technical publications are available for download from the BD Genomics Resource Library at [bd.com/genomics-resources](http://bd.com/genomics-resources).

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## Intended use

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### 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 \(page 18\)](#) and the [BD Rhapsody™ Scanner overview \(page 20\)](#).

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**Restrictions**

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

BD denies any responsibility for damage caused by the following:

- Any use of a BD Rhapsody™ 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

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**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.

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## Safety symbols

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### Introduction



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* (Doc ID: 42061).

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### Safety symbols






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

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

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**Other symbols**

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Symbol	Meaning
 The symbol consists of the letters 'REF' in a bold, sans-serif font, enclosed within a rectangular border.	Part number
 The symbol consists of the letters 'LOT' in a bold, sans-serif font, enclosed within a rectangular border.	Lot number
 A stylized icon of a thermometer, showing a vertical tube with a bulb at the bottom and a scale-like structure.	Storage temperature range
 A stylized icon of an hourglass, showing two inverted triangles meeting at a point in the middle.	Expiration date
 An icon of an open book with a lowercase letter 'i' on the right page, representing information or instructions.	Consult instructions for use

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## Safety data sheets

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### Introduction

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

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### Obtaining SDSs

Before handling chemicals, read and understand the SDSs. To obtain SDSs for chemicals ordered from BD Biosciences, go to [regdocs.bd.com](https://regdocs.bd.com), or contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).

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## Instrument technical support

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

**Contacting technical support** If technical assistance is required, contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) or 1.877.232.8995. You can contact technical support in Europe at [help.biosciences@europe.bd.com](mailto:help.biosciences@europe.bd.com) or at these telephone numbers:

Location	Telephone number	Location	Telephone number
Worldwide	+32 2 40 09 895	—	
Austria	01 92 80 465	Netherlands	010 71 14 800
Belgium	02 40 17 093	Norway	800 18 530
Denmark	80 88 21 93	Portugal	800 86 01 76
Finland	800 11 63 17	South Africa	0800 98 10 08
France	01 70 70 81 93	Spain	91 41 46 250
Germany	069 22 22 25 60	Sweden	08 50 69 21 54
Greece	00800 12 75 06	Switzerland	044 58 04 373
Italy	02 36 00 36 85	United Kingdom	0207 07 53 226

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
-



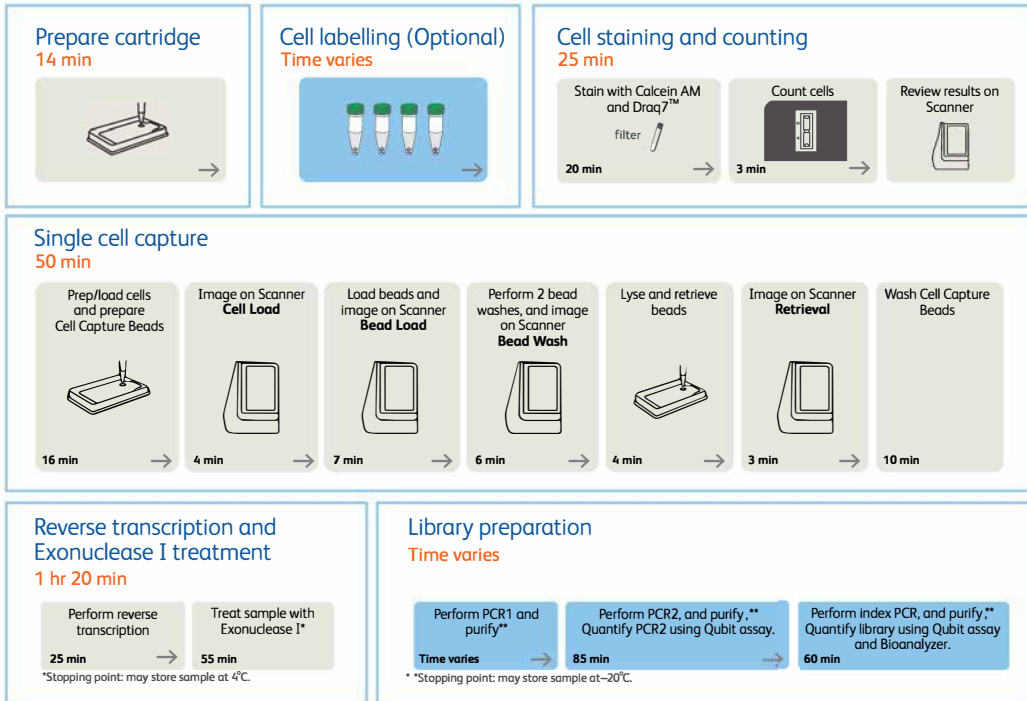
# 2

## Getting Started

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- [BD Rhapsody™ targeted library workflow \(page 16\)](#)
- [Site requirements \(page 17\)](#)
- [Thermomixer settings \(page 17\)](#)
- [Thermal cycler setup \(page 17\)](#)
- [Pipette Settings \(page 18\)](#)
- [BD Rhapsody™ Express instrument overview \(page 18\)](#)
- [BD Rhapsody™ Scanner overview \(page 20\)](#)
- [Quick guide to the BD Rhapsody Scanner software \(page 23\)](#)
- [Best practices \(page 27\)](#)

# BD Rhapsody™ targeted library workflow



To perform the workflow, follow the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

**Note:** For use of two cartridges, see [Workflow with two BD Rhapsody™ Cartridges](#) (page 131).

## Site requirements

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### Workspace designation

Dedicate two isolated workspaces 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* (Doc ID: 47391) and [BD Rhapsody™ Scanner metrics \(page 121\)](#).

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* (Doc ID: 43084).

Genomics technical publications are available for download from the BD Genomics Resource Library at [bd.com/genomics-resources](http://bd.com/genomics-resources).

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## Thermomixer settings

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### Settings

Depending on the protocol, set the thermomixer from 37°C to 80°C and 0–1,200 rpm.

## Thermal cycler setup

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### Recommendations

- Use a properly calibrated thermal cycler for 0.2 mL tubes with a maximum reaction volume of 50  $\mu$ L.
- Use a heated lid set to  $\geq 95^{\circ}\text{C}$ .
- Do not use fast cycling mode.

- For specific instrument operation, follow the instructions provided by the manufacturer.
- 

## Pipette Settings

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**Pipette programs** 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

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## BD Rhapsody™ Express instrument overview

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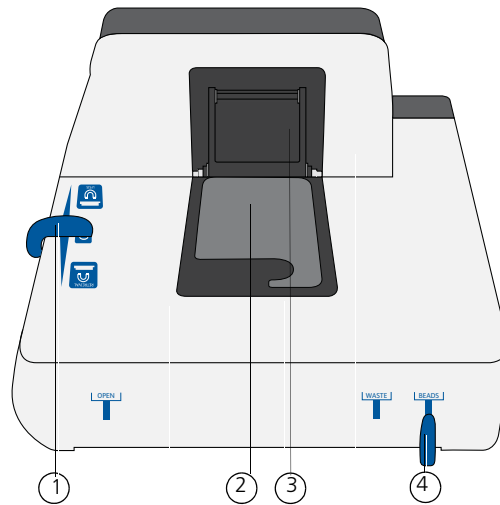
**Introduction** The BD Rhapsody Cartridge requires use of the BD Rhapsody Express instrument. The station is used to load reagents, cells, and Cell Capture Beads into the cartridge for bead capture and retrieval of single cell mRNA.

---

**Safety** For safety and limitations of the BD Rhapsody Express instrument, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner Safety and Limitations Guide* (Doc ID: 42061).

## Components

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* (Doc ID: 43084).



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) and 5 mL LoBind Tube for bead retrieval (BEADS) and for Waste Collection Container and 5 mL LoBind Tube access (OPEN)

## BD Rhapsody™ Scanner overview

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**Introduction** 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.

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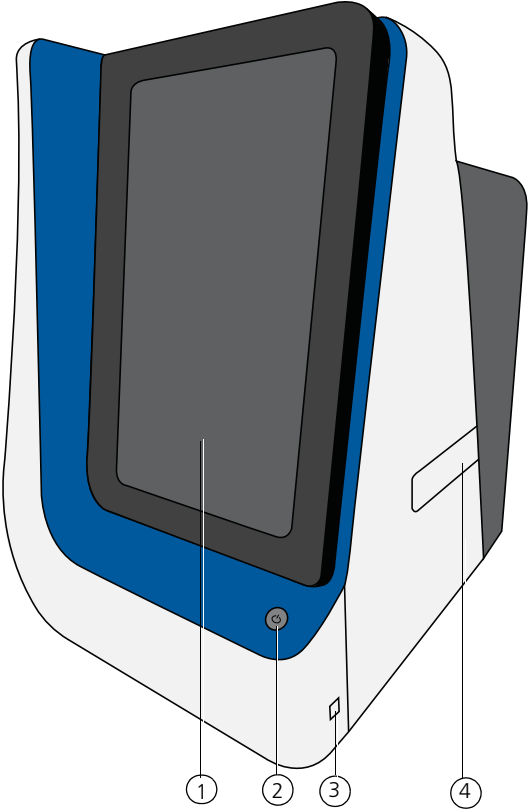
**Safety** For safety and limitations of the scanner, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner Safety and Limitations Guide* (Doc ID: 42061).

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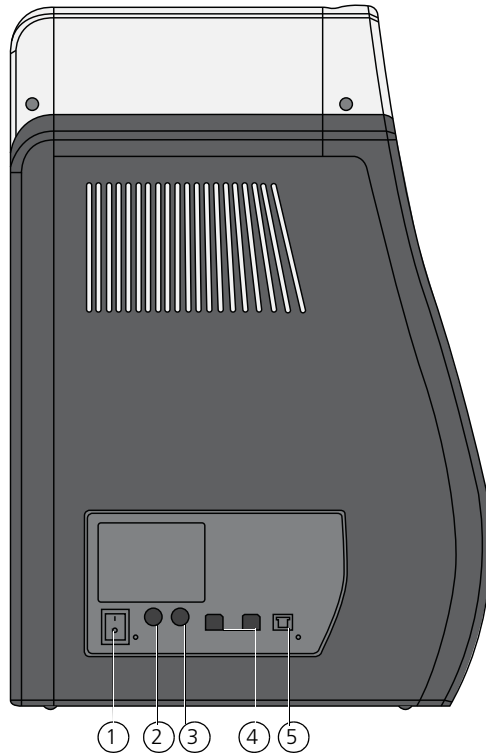
**Installation and maintenance** See the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide* (Doc ID: 43084) to review critical installation and periodic maintenance procedures on the scanner.

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**Components** 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



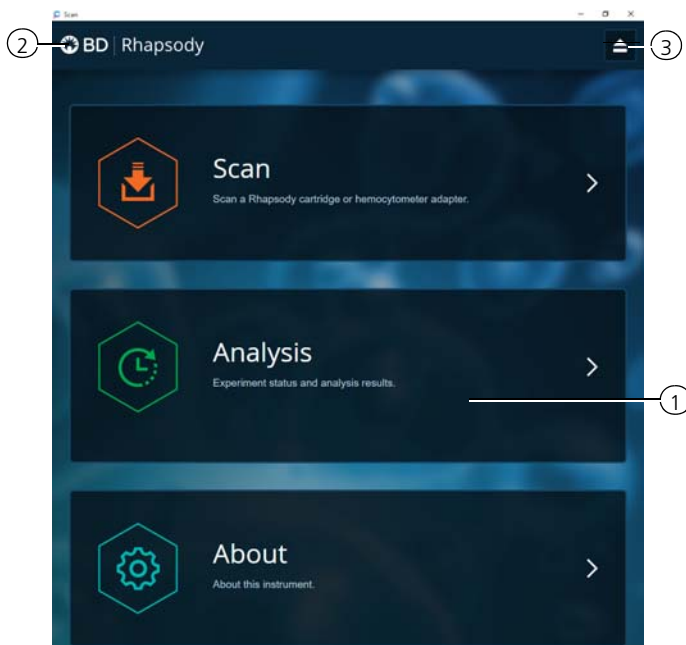
No.	Rear component
1	Master power switch
2	Fuse holder
3	24 VDC power input
4	USB 2.0 ports (2) <sup>a</sup>
5	Ethernet port

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



# Quick guide to the BD Rhapsody Scanner software

## Navigation



No.	Description
1	Tap an application (app) to navigate between scan, analysis, and about screens.
2	Tap <b>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.

The screenshot shows the 'Samples Calculator' interface in the BD Rhapsody software. The interface includes a top navigation bar with 'Scan', 'Experiment', and 'About' buttons. The main area is titled 'Samples Calculator' and contains several input fields and a table.

Callout 4 points to the 'Select Samples' dropdown menu. Callout 5 points to the 'Desired total volume' input field, which is set to 650  $\mu\text{l}$ . Callout 6 points to the 'Relative Amounts' column in the sample table. Callout 7 points to the 'Results' section, specifically the 'Stock Volume' column.

**Input Fields:**

- Cartridge type: 0109 (575  $\mu\text{l}$ )
- Desired total volume: 650  $\mu\text{l}$
- Desired number of captured cells: 10000 cells

**Sample Table:**

Sample	Time	Concentration (cells/ $\mu\text{l}$ )	Viable Cells (%)	Relative Amounts
Jurkat	2018-01-31 10:01:43.07	423.64	92.24	- 1 +
Ramos	2018-01-31 10:02:39.54	375.99	92.53	- 1 +

**Results Section:**

Sample	Stock Volume
Jurkat	15.3 $\mu\text{l}$
Ramos	17.2 $\mu\text{l}$

Other results shown:

- Buffer volume: 617.5  $\mu\text{l}$
- Loading cell concentration: 19.9 cells/ $\mu\text{l}$
- Estimated cell doublet rate: 2.4%

A green 'Save' button is located at the bottom of the interface.

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. Tap <b>Save</b> 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.

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**BD Rhapsody  
Scanner software  
workflows**

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**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

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## 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™ Single-Cell Analysis System Installation and Maintenance Guide* (Doc ID: 43084).
  - 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 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 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% bleach.
-

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**Sterility**

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

**Cell Capture Beads**

- Keep the Cell Capture Beads on ice before use.  
**Do not freeze Cell Capture Beads.**
  - For maximum recovery, do not vortex samples containing Cell Capture Beads.
  - Gently mix suspensions with Cell Capture Beads by pipette only.
  - Use low retention tips and LoBind tubes when handling Cell Capture Beads.
- 

**BD Rhapsody Express instrument**

- The Express instrument contains strong magnets. Avoid having metal pieces close to the station.
  - Wipe the 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 (Cat. No. 650000061) until ready to proceed with cell loading.
-

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**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.
-

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# 3

## Product information

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- Required kits and storage conditions (page 32)
- Suggested kits (page 41)
- Reagents (page 44)
- Consumables (page 47)
- Equipment (page 50)
- Software (page 54)

## Required kits and storage conditions

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### Introduction

The BD Rhapsody™ Targeted mRNA and AbSeq Reagent Kit—4 Pack (Cat. N. 633771) consists of four boxes:

- BD Rhapsody™ Cartridge Reagent Kit
- BD Rhapsody™ Cartridge Kit
- BD Rhapsody™ cDNA Kit
- BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit

The BD Rhapsody Targeted mRNA and AbSeq Amplification Kit (Cat. No. 633774) contains sufficient amplification reagents to prepare four libraries of each type, including targeted mRNA, Sample Tag, and BD® AbSeq libraries. Sub-sampling of the Exonuclease I-treated Cell Capture Beads might require purchasing additional BD Rhapsody Targeted mRNA and AbSeq Amplification kits.

To perform sample multiplexing with the BD Rhapsody™ system, one of the following additional kits are required:

- BD® Human Single-Cell Multiplexing Kit (Cat. No. 633781)
- BD® Mouse Immune Single-Cell Multiplexing Kit (Cat. No. 633793)

To perform antibody-oligonucleotide labelling, use additional these components:

- BD® AbSeq Ab-Oligos (antibody-oligonucleotides)
- BD Pharmingen™ Stain Buffer (FBS)
- (Optional) BD Pharmingen™ Human BD Fc Block™, for use with myeloid and B lymphocyte-containing samples

Product information on the kits and reagents are in this chapter.

**BD Rhapsody  
Targeted mRNA  
and AbSeq Reagent  
Kit—4 Pack (Cat.  
No. 633771)**

- Store the four kit boxes at the specified storage temperatures. Use only non-frost free freezers for reagent storage.
- Keep the reagents on ice unless instructed otherwise.
- The BD Rhapsody™ Cartridge is single-use only.
- Limit preparation of mixes to  $\leq 20\%$  overage.

Box	Component	Cap color	Quantity	Volume per unit	Storage
BD Rhapsody Cartridge Reagent Kit (Cat. No. 633731)	Cartridge Wash Buffer 1	Neutral	1 bottle	7 mL	2°C to 8°C
	Cartridge Wash Buffer 2	Neutral	1 bottle	4 mL	
	Sample Buffer	Neutral	1 bottle	28 mL	
	Lysis Buffer	Neutral	4 bottles	15 mL	
	Bead Wash Buffer	Neutral	1 bottle	10 mL	
	1 M DTT	White	1 vial	400 $\mu$ L	
	Cell Capture Beads	Brown	4 vials	2 mL	
Waste Collection Container	Neutral	4 each	—		

Box	Component	Quantity	Storage
BD Rhapsody Cartridge Kit (Cat. No. 633733)	BD Rhapsody Cartridge	4 each	15°C to 25°C

<b>Box</b>	<b>Component</b>	<b>Cap color</b>	<b>Quantity</b>	<b>Volume per unit</b>	<b>Storage</b>
BD Rhapsody cDNA Kit (Cat. No. 633773)	Nuclease-Free Water	Neutral	2 vials	1 mL	-25°C to -15°C
	RT Buffer	Orange	1 vial	200 µL	
	RT 0.1M DTT	Orange	1 vial	50 µL	
	Reverse Transcriptase	Orange	1 vial	50 µL	
	dNTP	Orange	1 vial	100 µL	
	RNase Inhibitor	Orange	1 vial	50 µL	
	Bead RT/PCR Enhancer	Black	1 vial	70 µL	
	10X Exonuclease I Buffer	Yellow	1 vial	100 µL	
	Exonuclease I	Yellow	1 vial	50 µL	
	Bead Resuspension Buffer	Black	1 vial	1 mL	

Box	Component	Cap color	Quantity	Volume per unit	Storage
BD Rhapsody Targeted mRNA and AbSeq Amplification Kit (Cat. No. 633774)	Nuclease-Free Water	Neutral	1 vial	1 mL	-25°C to -15°C
	Bead RT/PCR Enhancer	Black	1 vial	70 µL	
	PCR MasterMix	White	1 vial	1.2 mL	
	Elution Buffer	Pink	1 vial	1.8 mL	
	Universal Oligo	White	1 vial	130 µL	
	Library Forward Primer	Red	1 vial	40 µL	
	Library Reverse Primer 1	Red	1 vial	20 µL	
	Library Reverse Primer 2	Red	1 vial	20 µL	
	Library Reverse Primer 3	Red	1 vial	20 µL	
	Library Reverse Primer 4	Red	1 vial	20 µL	
	Bead Resuspension Buffer	Black	1 vial	1 mL	
	Sample Tag PCR1 Primer	Purple	1 vial	20 µL	
	Sample Tag PCR2 Primer	Purple	1 vial	20 µL	
	BD AbSeq Primer	Green	1 vial	70 µL	

Kit	Components	Quantity	Volume per unit	Storage
BD Human Single-Cell Multiplexing Kit (Cat. No. 633781)	Sample Tag 1— Human	1 vial	20 µL	2°C to 8°C
	Sample Tag 2— Human	1 vial	20 µL	
	Sample Tag 3— Human	1 vial	20 µL	
	Sample Tag 4— Human	1 vial	20 µL	
	Sample Tag 5— Human	1 vial	20 µL	
	Sample Tag 6— Human	1 vial	20 µL	
	Sample Tag 7— Human	1 vial	20 µL	
	Sample Tag 8— Human	1 vial	20 µL	
	Sample Tag 9— Human	1 vial	20 µL	
	Sample Tag 10— Human	1 vial	20 µL	
	Sample Tag 11— Human	1 vial	20 µL	
	Sample Tag 12— Human	1 vial	20 µL	

Kit	Components	Quantity	Volume per unit	Storage
BD Mouse Immune Single-Cell Multiplexing Kit (Cat. No. 633793)	Sample Tag 1— Mouse Immune	1 vial	20 µL	2°C to 8°C
	Sample Tag 2— Mouse Immune	1 vial	20 µL	
	Sample Tag 3— Mouse Immune	1 vial	20 µL	
	Sample Tag 4— Mouse Immune	1 vial	20 µL	
	Sample Tag 5— Mouse Immune	1 vial	20 µL	
	Sample Tag 6— Mouse Immune	1 vial	20 µL	
	Sample Tag 7— Mouse Immune	1 vial	20 µL	
	Sample Tag 8— Mouse Immune	1 vial	20 µL	
	Sample Tag 9— Mouse Immune	1 vial	20 µL	
	Sample Tag 10— Mouse Immune	1 vial	20 µL	
	Sample Tag 11— Mouse Immune	1 vial	20 µL	
	Sample Tag 12— Mouse Immune	1 vial	20 µL	

**BD Rhapsody™  
targeted primer  
panels**

Each panel includes a set of primers designed to target human (Hs) or mouse (Mm) genes. Each panel contains sufficient primers to prepare four libraries.

A supplement to a panel can be designed based on individual needs. The BD Rhapsody™ Panel Supplement contains primer pairs for additional targeted sequencing of selected genes. For more information on BD Rhapsody Panel Supplements, contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) and see [BD Rhapsody™ Panel Supplement \(Cat. No. 633742\)](#) (page 42).

Panel	Component	Cap color	Quantity	Vol. (µL)	Storage
BD Rhapsody™ Immune Response Panel Hs (Cat. No. 633750)	PCR1 Primers-Immune Response Panel Hs (tube label: PCR1-Immune Res. Hs)	Blue	1 vial	210	-25°C to -15°C
	PCR2 Primers-Immune Response Panel Hs (tube label: PCR2-Immune Res. Hs)	Blue	1 vial	50	
BD Rhapsody™ T Cell Expression Panel Hs (Cat. No. 633751)	PCR1 Primers-T Cell Expression Panel Hs (tube label: PCR1-T Cell Expression Hs)	Blue	1 vial	210	-25°C to -15°C
	PCR2 Primers-T Cell Expression Panel Hs (tube label: PCR2-T Cell Expression Hs)	Blue	1 vial	50	
BD Rhapsody™ Onco-BC Panel Hs (Cat. No. 633752)	PCR1 Primers-Onco-BC Panel Hs (tube label: PCR1-Onco-BC Hs)	Blue	1 vial	210	-25°C to -15°C
	PCR2 Primers-Onco-BC Panel Hs (tube label: PCR2-Onco-BC Hs)	Blue	1 vial	50	



Panel (continued)	Component	Cap color	Quantity	Vol. (µL)	Storage
BD Rhapsody™ Immune Response Panel Mm (Cat. No. 633753)	PCR1 Primers-Immune Response Panel Mm (tube label: PCR1-Immune Res. Mm)	Blue	1 vial	210	-25°C to -15°C
	PCR2 Primers-Immune Response Panel Mm (tube label: PCR2-Immune Res. Mm)	Blue	1 vial	50	

**BD Rhapsody™  
Targeted mRNA  
and AbSeq Training  
Kit—4 Pack (Cat.  
No. 633772)**

For detailed information on the kit components, see [BD Rhapsody Targeted mRNA and AbSeq Reagent Kit—4 Pack \(Cat. No. 633771\)](#) (page 33).

Component	Quantity	Storage
BD Rhapsody Cartridge Reagent Kit (Cat. No. 633731)	1 kit	2°C to 8°C
BD Rhapsody Cartridge Kit (Cat. No. 633733)	1 kit	15°C to 25°C
BD Rhapsody cDNA Kit (Cat. No. 633773)	1 kit	-25°C to -15°C
BD Rhapsody Targeted mRNA and AbSeq Amplification Kit (Cat. No. 633774)	1 kit	
BD Rhapsody Immune Response Panel Hs (Cat. No. 633750)	1 kit	

### Required kits from other vendors

Kit	Supplier	Catalog no.
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851

## Suggested kits

**BD Rhapsody™ Custom Panel** The BD Rhapsody Custom Panel contains a maximum of 500 custom primers. To order a BD Rhapsody Custom Panel, contact BD Biosciences at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).

Panel	Component	Cap color	Quantity	Volume	Storage
BD Rhapsody Custom Panel (U.S. only: Cat. No. 633743)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Custom Panel 2–99 genes (Outside U.S.: Cat. No. 633777)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Custom Panel 100–199 genes (Outside U.S.: Cat. No. 633778)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

Panel	Component	Cap color	Quantity	Volume	Storage
BD Rhapsody Custom Panel 200–299 genes (Outside U.S.: Cat. No. 633779)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Custom Panel 300–399 genes (Outside U.S.: Cat. No. 633783)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Custom Panel 400–500+ genes (Outside U.S.: Cat. No. 633784)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	1.4 mL <sup>b</sup>	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx <sup>a</sup>	Blue	1 vial	350 µL <sup>b</sup>	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

- Each design has a unique identifier that is provided with your BD Rhapsody Custom Panel primer design.
- To prepare a 1X dilution for use in the assay, dilute 1 part PCR primer to 9 parts of IDTE buffer. Store the 1X dilution at –25°C to –15°C for ≤2 years.

**BD Rhapsody™  
Panel Supplement  
(Cat. No. 633742)**

Add up to 100 additional supplemental primers to the BD Rhapsody targeted (predesigned) or custom panels for a maximum of 500 primers that can be used in an experiment. To order a BD Rhapsody Panel Supplement, contact BD Biosciences at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com).

Up to two BD Rhapsody Panel Supplements can be added per reaction provided that the total number of primers added is  $\leq 500$  (predesigned or custom panel plus panel supplement) and the primers have been designed to be compatible.

Panel	Component	Cap color	Quantity	Volume	Storage
BD Rhapsody Panel Supplement (U.S. only: Cat. No. 633742)	BD Rhapsody™ 10X PCR1 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	120 $\mu\text{L}^b$	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	30 $\mu\text{L}^b$	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Panel Supplement 2–49 genes (Outside U.S.: Cat. No. 633770)	BD Rhapsody™ 10X PCR1 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	120 $\mu\text{L}^b$	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	30 $\mu\text{L}^b$	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Panel Supplement 50–100 genes (Outside U.S.: Cat. No. 633776)	BD Rhapsody™ 10X PCR1 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	120 $\mu\text{L}^b$	-25°C to -15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx <sup>a</sup>	Blue	1 vial	30 $\mu\text{L}^b$	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

- Each design has a unique identifier that is provided with your BD Rhapsody Panel Supplement primer design.
- To prepare a 1X dilution for use in the assay, dilute 1 part PCR primer to 9 parts of IDTE buffer. Store the 1X dilution at -25°C to -15°C for  $\leq 2$  years.

### Suggested kits from other vendors

Reagent	Supplier	Catalog no.
Agilent DNA High Sensitivity Kit	Agilent Technologies	5067-4626
High Sensitivity D1000 ScreenTape	Agilent Technologies	5067-5584
High Sensitivity D1000 Reagents	Agilent Technologies	5067-5585

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## Reagents

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### Required reagent for BD Rhapsody Cartridge workflow

Reagent	Supplier	Catalog no.
Absolute ethyl alcohol, molecular biology grade	Major supplier	—
Nuclease-free water	Major supplier	—

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### Required reagents for sample multiplexing and/or antibody- oligonucleotide labelling

Material	Supplier	Catalog no.
BD Pharmingen Stain Buffer (FBS)	BD Biosciences	554656
BD AbSeq Ab-Oligos	BD Biosciences	Various

### Required reagents for cell preparation and staining

Reagent	Supplier	Catalog no.
Calcein AM cell-permeant dye <sup>a</sup>	Thermo Fisher Scientific	C1430
DRAQ7™ <sup>a</sup> , 0.3 mM	BD Biosciences	564904
Dimethyl sulfoxide (DMSO)	Major supplier	—
70% ethyl alcohol or 70% isopropyl alcohol <sup>b</sup>	Major supplier	—

- 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* (Doc ID: 43084). Instead of 70% alcohol, 10% (v/v) bleach can be used.

### Required reagents for PCR product purification

Reagent	Supplier	Catalog no.
Agencourt® AMPure® XP magnetic beads	Beckman Coulter	A63880
Ethyl alcohol, Pure (200 proof, molecular biology grade)	Sigma-Aldrich	E7023-500ML

**Suggested reagent  
for cell suspension  
preparation**

Reagent	Supplier	Catalog no.
1X RBC Lysis Buffer	Thermo Fisher Scientific	00-4333-57
Phosphate-buffered saline (calcium- and magnesium-free)	Major supplier	—

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**Suggested reagents  
for sample  
multiplexing and/or  
antibody-  
oligonucleotide  
labelling**

Material	Supplier	Catalog no.
BD Pharmingen Human BD Fc Block <sup>a</sup>	BD Biosciences	564220

- a. For use with myeloid and B lymphocyte-containing samples.
-



## Consumables

### Required consumables

Consumable	Supplier	Catalog no.
Falcon® Tube with Cell Strainer Cap	Corning	352235
Falcon tubes, 5 mL Round Bottom Polystyrene Test Tube <sup>a</sup>	Corning	352054
Improved Neubauer Hemocytometer	INCYTO	DHC-N01-5
DNA LoBind Tubes, 1.5 mL <sup>b</sup>	Eppendorf	0030108051
DNA LoBind Tubes, 5 mL  <b>Note:</b> These are the Bead Retrieval Tubes to be used with the BD Rhapsody Express instrument.	Eppendorf	0030108310
Low retention filtered pipette tips, 10 µL <sup>b</sup>	Major supplier	—
Low retention filtered pipette tips, 200 µL <sup>b</sup>	Major supplier	—
Low retention filtered pipette tips, 1,000 µL <sup>b</sup>	Major supplier	—

Consumable (continued)	Supplier	Catalog no.
Gilson™ PIPETMAN™ Tipack™ Filter Tips, 100–1,200 µL for BD Rhapsody™ P1200M pipette	Thermo Fisher Scientific	F171803G
Gilson PIPETMAN Tipack Racked Pipet Tips, 500–5,000 µL for BD Rhapsody™ P5000M pipette	Thermo Fisher Scientific	F161370G
Qubit™ Assay Tubes	Thermo Fisher Scientific	Q32856
Empty Latch Racks for 500 µL Tubes <sup>c</sup>	Thermo Fisher Scientific	4900 (4890 is also acceptable)
0.2 mL PCR 12-strip tubes <sup>a</sup>	Major supplier	—
10 mL sterile serological pipettes	Major supplier	—
Premoistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol	Major supplier	—
Lint-free wipers	Major supplier	—

- a. Required for sample multiplexing and/or antibody-oligonucleotide labelling.
- b. Provide material in both pre- and post-amplification workspaces.
- c. Required for storing tubes of BD® AbSeq Ab-Oligos and convenient pooling of reagents.

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**Suggested  
consumables**

Consumable item	Supplier	Catalog no.
BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube– Sodium Heparin <sup>a</sup>	BD Biosciences	362753

- a. For single cell preparation of peripheral blood mononuclear cells (PBMCs).
-

## Equipment

### Required equipment

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

Equipment	Box	Components
BD Rhapsody™ Single-Cell Analysis system	BD Rhapsody Scanner (Cat. No. 633701)	<ul style="list-style-type: none"> <li>• BD Rhapsody Scanner</li> <li>• Power supply and cable</li> <li>• Hemocytometer Adapter (Cat. No. 633703)</li> <li>• BD Rhapsody P1200M pipette (Cat. No. 633704)</li> <li>• BD Rhapsody P5000M pipette (Cat. No. 633705)</li> </ul>
	BD Rhapsody Express instrument (Cat. No. 633702)	<ul style="list-style-type: none"> <li>• BD Rhapsody Express instrument</li> </ul>

Equipment	Supplier	Catalog no.
6-Tube Magnetic Separation Rack for 1.5 mL tubes <sup>a</sup>	New England Biolabs	S1506S
Large magnetic separation stand	V&P Scientific, Inc.	VP 772FB-1
Clear acrylic cylinder adapter for 15 mL tube magnet <sup>b</sup>	V&P Scientific, Inc.	VP 772FB-1A
Low-profile magnetic separation stand for 0.2 mL, 8-strip tubes	V&P Scientific, Inc. OR	VP 772F4-1
	Clontech	635011
Thermomixer (37°C, 1,200 rpm): <ul style="list-style-type: none"> <li>• ThermoMixer® C</li> <li>• SmartBlock™ Thermoblock 1.5 mL</li> </ul>	Eppendorf	<ul style="list-style-type: none"> <li>• 5382000023</li> <li>• 5360000038</li> </ul>
Qubit™ 3.0 Fluorometer	Thermo Fisher Scientific	Q33216
Heat block capable of 80°C Suggested: VWR® Advanced Mini Dry Block Heater with Heated Lid	VWR	10153-348

Equipment (continued)	Supplier	Catalog no.
<ul style="list-style-type: none"> <li>• 2100 Bioanalyzer OR</li> <li>• 4200 TapeStation Instrument</li> </ul>	Agilent Technologies	<ul style="list-style-type: none"> <li>• G2940CA</li> <li>• G2991AA</li> </ul>
Thermal cycler with heated lid	Major supplier	—
Water bath OR incubator at 37°C	Major supplier	—
Laminar flow hood	Major supplier	—
Digital timer <sup>a</sup>	Major supplier	—
Pipettes (P10, P20, P200, P1000) <sup>a</sup>	Major supplier	—
Multi-channel pipette, 2–20 µL OR 20–200 µL <sup>a</sup>	Major supplier	—
Microcentrifuge for 1.5–2.0 mL tubes <sup>a</sup>	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	—
Vortexer <sup>a</sup>	Major supplier	—
Pipet-Aid	Major supplier	—

- a. Provide material in both pre- and post-amplification workspaces.  
b. Holds 5 mL LoBind Tube in magnet.

## 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 <sup>a</sup>	Major supplier	—
Phase-contrast microscope	Major supplier	—
8-Channel Screw Cap Tube Capper <b>Note:</b> Optional for antibody-oligonucleotide labelling	Thermo Fisher Scientific	4105MAT

- a. The external barcode reader is optional. The scanner has an internal barcode reader. To purchase an external barcode reader, BD Biosciences recommends the USB Automatic Barcode Scanner Scanning Barcode Reader with Hands-Free Adjustable Stand (Brainydeal) or equivalent.

## Software

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### **BD Rhapsody™ Analysis pipeline**

The BD Rhapsody Analysis pipeline takes the FASTQ read files and reference files for gene alignment. The pipeline filters by read quality, annotates R1 and R2 reads, annotates molecules, determines putative cells, determines the sample of origin (sample multiplexing only), generates expression matrices, generates a metrics summary, and performs clustering analysis.

For installation, see the *BD Single Cell Genomics Analysis Setup User Guide* (Doc ID: 47383).

For detailed information on the BD Rhapsody Analysis pipeline, see the *BD Single Cell Genomics Bioinformatics Handbook* (Doc ID: 54169).

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### **BD® Data View**

BD Data View is a software tool for visualization and exploratory analysis of output files generated following bioinformatics analysis. The software is included with the purchase of the BD Rhapsody™ Single-Cell Analysis system and is optional for use.

For installation, see the *BD Single Cell Genomics Analysis Setup User Guide* (Doc ID: 47383).

For detailed instructions on using BD Data View, see the *BD Single Cell Genomics Bioinformatics Handbook* (Doc ID: 54169).

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# 4

## **Preparing the BD Rhapsody™ Cartridge**

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- Priming the BD Rhapsody Cartridge (page 58)
- Treating the surface of the cartridge (page 65)

## Priming the BD Rhapsody Cartridge

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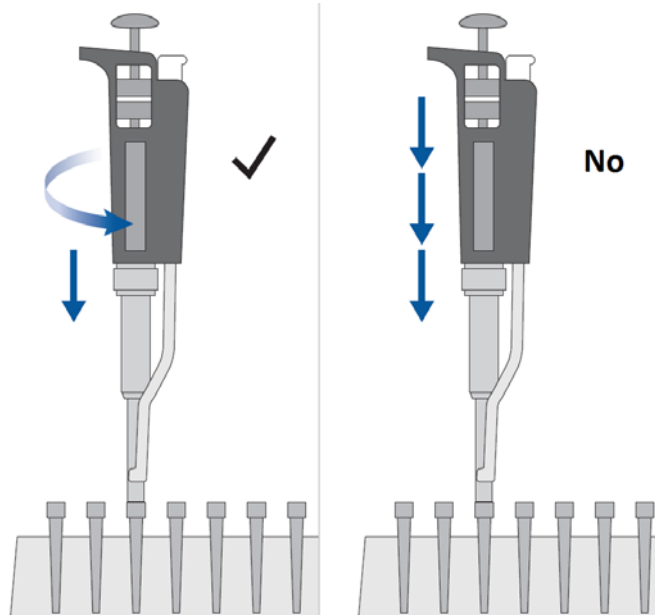
**Before you begin** After opening the cartridge packet, ensure that you prepare the cartridge in  $\leq 1$  day. If you leave the cartridge at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ , equilibrate to room temperature ( $15^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ) for 5 minutes.

If cell preparation takes  $\geq 4$  hours, begin preparing cells before cartridge preparation. See the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524) to find the appropriate protocol to follow.

- Equilibrate these reagents at room temperature ( $15^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ) for  $\geq 30$  minutes before use:
    - Cartridge Wash Buffer 1 (Cat. No. 650000060)
    - Cartridge Wash Buffer 2 (Cat. No. 650000061)
  - Place these reagents on ice:
    - Sample Buffer (Cat. No. 650000062)
    - 1 M DTT (Cat. No. 650000063)
    - Lysis Buffer (Cat. No. 650000064)
    - DRAQ7 (protected from light)
  - Once at room temperature ( $15^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ), resuspend Calcein AM (1 mg; Thermo Fisher Scientific Cat. No. C1430) in 503.0  $\mu\text{L}$  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 \(page 18\)](#).
  - For the use of two cartridges, see [Workflow with two BD Rhapsody™ Cartridges \(page 131\)](#).
-

**Procedure**

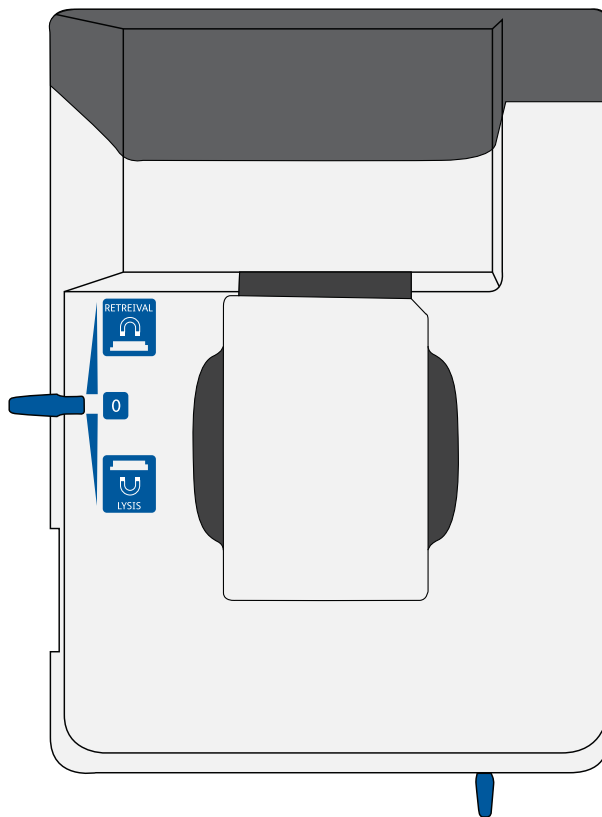
To ensure an air-tight seal with the BD Rhapsody™ P1200M or P5000M pipette, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft:



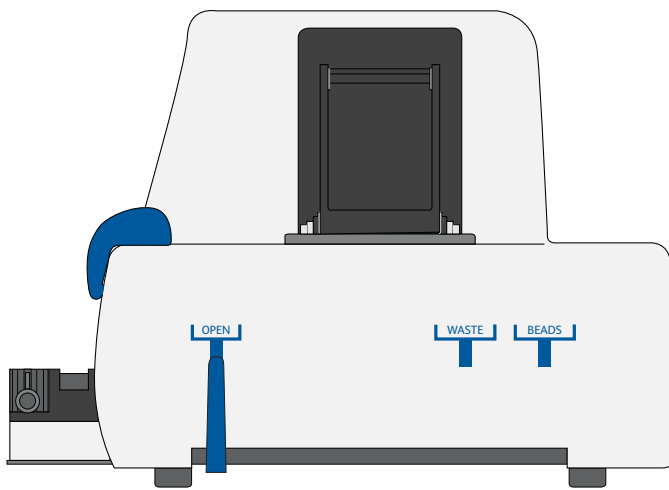
Avoid introducing bubbles while pipetting into the BD Rhapsody™ Cartridge.

**Change pipette tips before every pipetting step.**

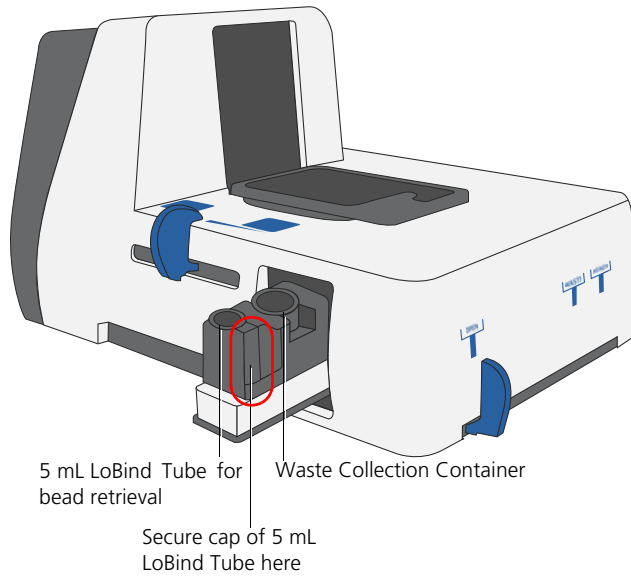
1. Move the left slider to the middle (0) position on the Express instrument. The Retrieval (top) magnet and Lysis (bottom) magnets are away from the cartridge tray:



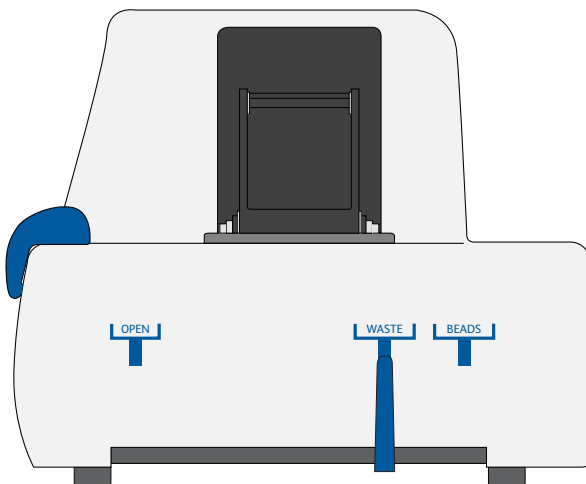
2. Move the front slider to **OPEN**:



3. Remove the cap of a Waste Collection Container (Cat. No. 650000090), and insert both the container and a new 5 mL LoBind Tube (Eppendorf Cat. No. 0030108310) for bead retrieval into the appropriate slots in the drawer. Secure the cap of the 5 mL LoBind Tube to the holder:

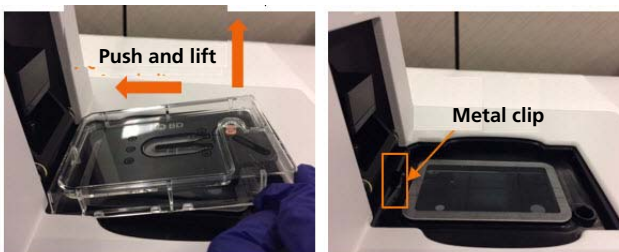


4. Move the front slider to **WASTE**:



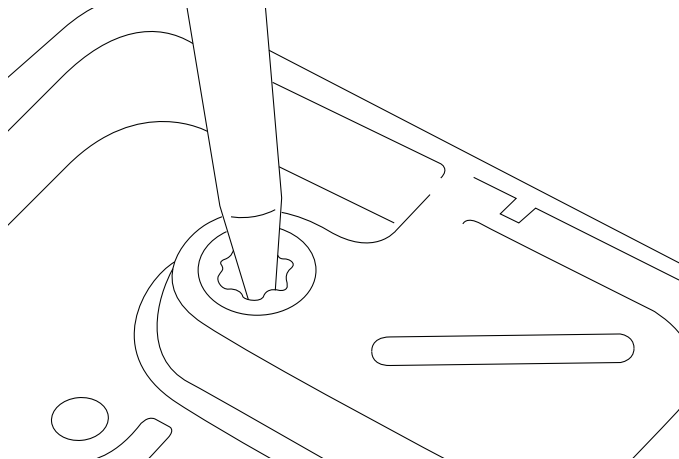
5. Push the cartridge into the far end of the tray to match cartridge and tray notches. Lay the cartridge flat, and release it. Ensure that the cartridge is flat in the tray and the barcode faces out.

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





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  $\mu\text{L}$ , and press the button again to dispense 700  $\mu\text{L}$ .

6. Load the cartridge with 700  $\mu\text{L}$  of 100% (absolute) ethyl alcohol using the P1200M pipette in **Prime/Treat** mode.
  7. Load the cartridge with 700  $\mu\text{L}$  of air using the P1200M pipette in **Prime/Treat** mode.
  8. Load the cartridge with 700  $\mu\text{L}$  of Cartridge Wash Buffer 1 (Cat. No. 650000060) with the P1200M pipette in **Prime/Treat** mode.
  9. Leave the cartridge on the tray at room temperature (15°C to 25°C) for 1 minute.
-

## Treating the surface of the cartridge

---

### Procedure

1. Load the cartridge with 700  $\mu$ L of air using the P1200M pipette in **Prime/Treat** mode.
2. Load the cartridge with 700  $\mu$ L of Cartridge Wash Buffer 1 (Cat. No. 650000060) using the P1200M pipette in **Prime/Treat** mode.
3. Leave the cartridge on the tray at room temperature (15°C to 25°C) for 10 minutes.
4. Load the cartridge with 700  $\mu$ L of air using the P1200M pipette in **Prime/Treat** mode.
5. Load the cartridge with 700  $\mu$ L of Cartridge Wash Buffer 2 (Cat. No. 650000061) using the P1200M pipette in **Prime/Treat** mode.

**Stopping point:** The cartridge can be stored at room temperature (15°C to 25°C) for  $\leq$ 4 hours. You can leave the cartridge on the tray. The performance of the cartridge has not been validated at room temperature (15°C to 25°C) storage for  $>$ 4 hours.

6. Prepare a single cell suspension. See the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524) to find the appropriate protocol to follow.
-

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# 5

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

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- Best practices for cell handling and cell counting (page 68)
- Staining cells with viability markers (page 69)
- Loading cells into the Hemocytometer Adapter (page 70)
- Counting cells with the BD Rhapsody Scanner (page 72)
- Preparing a cell suspension and loading cells in the BD Rhapsody™ Cartridge (page 79)
- Imaging cells in a cartridge (page 89)
- Preparing Cell Capture Beads (page 93)
- Loading Cell Capture Beads and imaging (page 94)
- Lysing cells and retrieving Cell Capture Beads (page 95)
- Performing reverse transcription on the Cell Capture Beads (page 101)
- Treating the sample with Exonuclease I (page 106)

## Best practices for cell handling and cell counting

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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, 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. Visually check filtered cell suspensions for debris.
  - Avoid pipetting low volumes of cells (<2  $\mu$ L).
  - Cell counting the BD Rhapsody™ Scanner is most accurate when the cell concentration is in the range of ~200–800 cells/ $\mu$ L. If the cell concentration is >1,000 cells/ $\mu$ L, dilute the cell suspension in cold Sample Buffer (Cat. No. 650000062) to ~200–800 cells/ $\mu$ L.
  - 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

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- Before you begin**
- Prepare a single cell suspension. See the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524) to find the appropriate protocol to follow.  
**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* (Doc ID: 210964).**
  - Thaw Calcein AM. Once at room temperature (15°C to 25°C), resuspend Calcein AM (1 mg; Thermo Fisher Scientific Cat. No. C1430) 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 (Cat. No. 650000062), 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  $\leq 30,000$  cells in 610 µL, proceed to step 2.

**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 (Thermo Fisher Scientific Cat. No. C1430) and 3.1 µL of 0.3 mM DRAQ7™ (BD Biosciences Cat. No. 564904) to 620 µL cell suspension (1:200 dilution) in cold Sample Buffer (Cat. No. 650000062).
3. Gently pipet-mix.
4. Incubate at 37°C in dark for 5 minutes.

5. Filter cells through Falcon® Tube with Cell Strainer Cap (Corning 352235).

**For low abundance or low volume samples, filtering is optional at this step. BD Biosciences recommends filtering the final sample before loading cells into the cartridge.**

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

## Loading cells into the Hemocytometer Adapter

---

**Before you begin**      Stain cells with viability markers. See [Staining cells with viability markers \(page 69\)](#).

**Count cells immediately.**

---

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

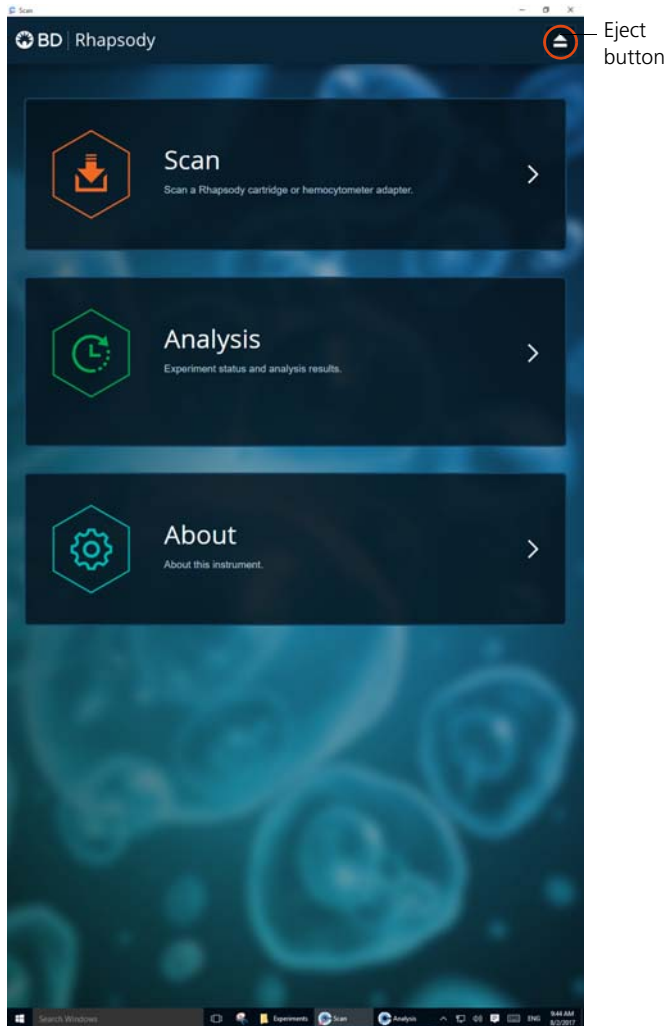
1. Gently mix cells well by pipette, and then gently pipet 10 µL of the cell suspension into one chamber of the INCYTO™ disposable hemocytometer (INCYTO Cat. No. DHC-N01-5).

2. Insert the hemocytometer into the Hemocytometer Adapter (Cat. No. 633703) so that the A and B sides of the hemocytometer align with A and B on the Hemocytometer Adapter.
  3. Wait for 1 to  $\leq 5$  minutes after loading.
  4. Count the cells in the BD Rhapsody™ Scanner.
-



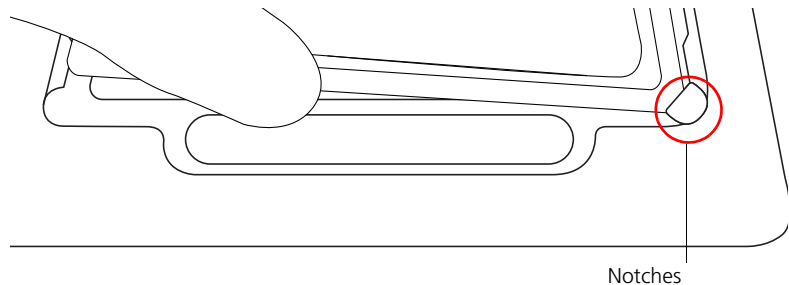
# Counting cells with the BD Rhapsody Scanner

- Procedure**
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@bdscmix.bd.com](mailto:scomix@bdscmix.bd.com).
  - To troubleshoot scanner software error messages, see [BD Rhapsody Scanner software messages \(page 116\)](#).
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.)

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

For example:

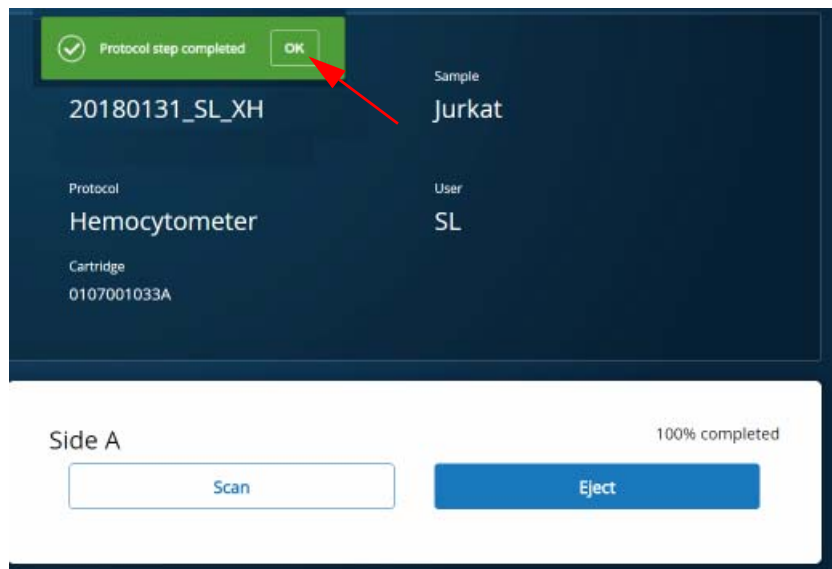
The screenshot displays the instrument's configuration interface. It features a white background with a dark blue border. At the top, there are five input fields arranged in two columns. The first column contains 'Experiment' (a dropdown menu with '20180131\_SL\_XH' selected), 'Protocol' (a dropdown menu with 'Hemocytometer' selected), and 'Cartridge' (a text field with '0107001033A'). The second column contains 'Sample' (a dropdown menu with 'Jurkat' selected) and 'User' (a text field with 'SL'). Below these fields, the interface is divided into two sections: 'Side A' and 'Side B'. The 'Side A' section has a white background and a blue button labeled 'Start Side A Scan'. The 'Side B' section has a dark blue background and is currently empty.

**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.

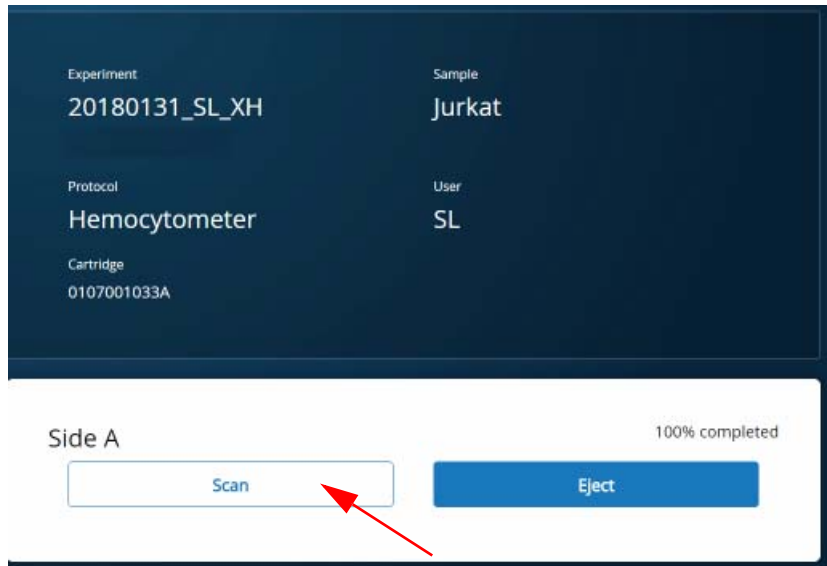
6. Tap **Side A** or **Side B**, depending on which side the cells were loaded, and then Select **Start Side A Scan** or **Start Side B Scan**, as required.

**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**:



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:

The screenshot displays two panels, one for the 'Jurkat' sample and one for the 'Ramos' sample. Each panel includes a header with the sample name and hemocytometer ID, a table of scan details, and an 'Analysis' section. The 'Analysis' sections are highlighted with red boxes.

**Jurkat**  
Hemocytometer 0107001033A

Scan Date	Sample	Step	Analysis Status
2018-01-31 10:01:43.07	Jurkat	Side A	✓ Completed

**Analysis**

Total cell concentration (live and dead)	423.64
Cell viability	92.24

**Ramos**  
Hemocytometer 0107001033A

Scan Date	Sample	Step	Analysis Status
2018-01-31 10:02:39.54	Ramos	Side B	✓ Completed

**Analysis**

Total cell concentration (live and dead)	375.99
Cell viability	92.53

**Note:** If no value could be calculated, then NaN (not a number) is displayed. See [Troubleshooting \(page 109\)](#).

12. Proceed as follows:
  - If the cell concentration is  $\leq 1,000$  cells/ $\mu$ L, proceed to step [13](#).
  - If the cell concentration is  $> 1,000$  cells/ $\mu$ L, dilute the cell suspension in cold Sample Buffer (Cat. No. 650000062) to  $\sim 200$ – $800$  cells/ $\mu$ 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 \(page 79\)](#).

**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 pre-amplification workspace.
  - Prepare cells as close to cell loading as possible. Keep other reagents, including Sample Buffer (Cat. No. 650000062) 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 \(page 57\)](#).
  - Thaw reagents (not enzymes) in the BD Rhapsody™ cDNA Kit at room temperature (15°C to 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 (Cat. No. 650000062)
    - 1 M DTT (Cat. No. 650000063)
    - Lysis Buffer (Cat. No. 650000064)
    - Cell Capture Beads (Cat. No. 650000089)
- 

### Loading cells into the cartridge

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 Cell Capture Beads:



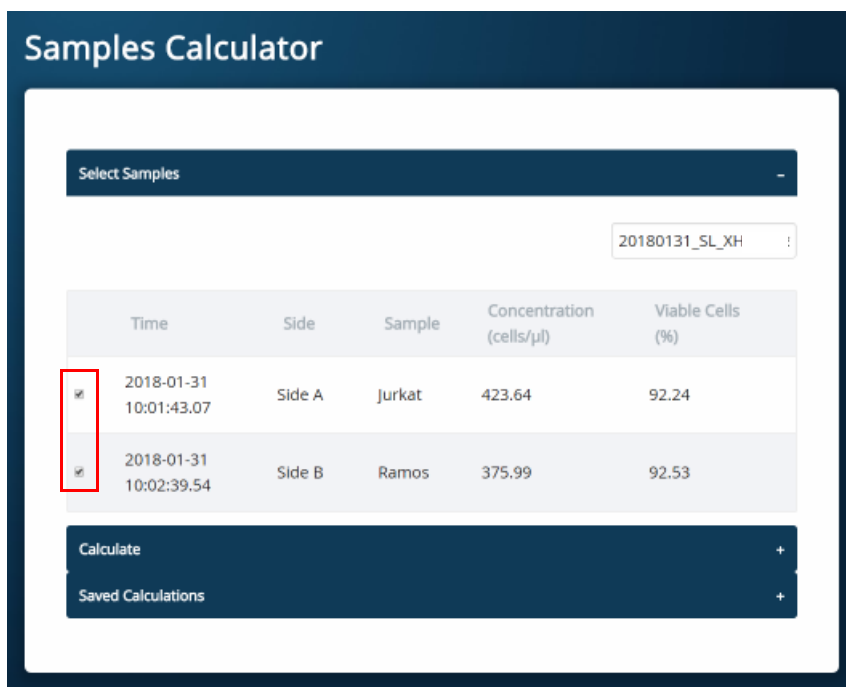
**Estimated multiplet rate based on the number of captured cells on retrieved Cell Capture Beads**

<b>Number of captured cells on retrieved Cell Capture Beads (target)<sup>a,b</sup></b>	<b>Estimated multiplet rate (%)</b>
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

Number of captured cells on retrieved Cell Capture Beads (target) <sup>a,b</sup> (continued)	Estimated multiplet rate (%)
18,000	4.2
19,000	4.5
20,000	4.7

- a. The number of cells sequenced might be less than the number of cells captured due to bead loss during handling, panel choice, and sample composition. The validated range of cells sequenced is 100–10,000 cells.
  - 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 stock cell and volume of cold Sample Buffer (Cat. No. 650000062) 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 check boxes to de-select samples. 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  $\mu\text{L}$  of a mixture of two samples and 10,000 cells captured are entered:

### Samples Calculator

Select Samples +

Calculate -

Cartridge type  
0109 575  $\mu\text{l}$

Desired total volume - 650 +  $\mu\text{l}$

Desired number of captured cells - 10000 + cells

Sample	Time	Concentration (cells/ $\mu\text{l}$ )	Viable Cells (%)	Relative Amounts
--------	------	--	---------------------	------------------

- 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/ $\mu$ 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 µl
Ramos	17.2 µl
Buffer volume	617.5 µl
Loading cell concentration	19.9 cells/µl
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.

1. (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 $\mu$ l	Buffer volume	617.5 $\mu$ l
Desired total volume	650 $\mu$ l	Loading cell concentration	19.9 cells/ $\mu$ l
Desired number of captured cells	10000 cells	Estimated cell doublet rate	2.4 %

Sample	Operator	Scan Time	Concentration (cells/ $\mu$ 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 <b>Stock Volume</b> is <610 $\mu\text{L}$	In a new 1.5 mL LoBind Tube, prepare the cell suspension in cold Sample Buffer (Cat. No. 650000062) according to the volumes obtained in step 3.
<ul style="list-style-type: none"> <li>• Calculated <b>Stock Volume</b> <math>\geq 610 \mu\text{L}</math>, or</li> <li>• <b>Buffer volume</b> is negative, or</li> <li>• <b>Cell concentration too low</b> warning is displayed</li> </ul>	Use 610 $\mu\text{L}$ of the cold cell suspension for cartridge cell loading. <sup>a</sup>

- 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  $\mu\text{L}$ . For example, if the Samples Calculator specifies mixing 700  $\mu\text{L}$  of Jurkat cells and 30  $\mu\text{L}$  of Ramos cells, mix 580  $\mu\text{L}$  of Jurkat cells with 30  $\mu\text{L}$  of Ramos cells.

**Ensure the stock solution is well suspended by gently pipetting cells up and down before transferring the appropriate volume for dilution.**

5. 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 (Thermo Fisher Scientific Cat. No. 352235) before loading the sample into the cartridge.
6. Load the cartridge on the tray with 700  $\mu\text{L}$  of air using the BD Rhapsody P1200M pipette in **Prime/Treat** mode.
7. Change the mode of the BD Rhapsody P1200M pipette to **Cell Load**.



8. With a manual pipette, gently pipet the cell suspension up and down to mix.
9. On the BD Rhapsody P1200M pipette, press the pipette button once to aspirate 40  $\mu\text{L}$  of air, immerse the pipette tip in cell suspension, and then press the button again to aspirate 575  $\mu\text{L}$  of cold cell suspension.
10. 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  $\mu\text{L}$  of air and cells.

**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™ Express instrument: Leave the cartridge with loaded cells on the tray at room temperature (15°C to 25°C) for 15 minutes. During incubation on the laboratory bench, prepare the Cell Capture Beads (Cat. No. 650000089). See [Preparing Cell Capture Beads \(page 93\)](#). After preparing the Cell Capture Beads, proceed to [Imaging cells in a cartridge](#), or
    - In the scanner: Proceed immediately to [Imaging cells in a cartridge \(page 89\)](#) to install the cartridge and enter a 15 minute run delay.
-

## Imaging cells in a cartridge

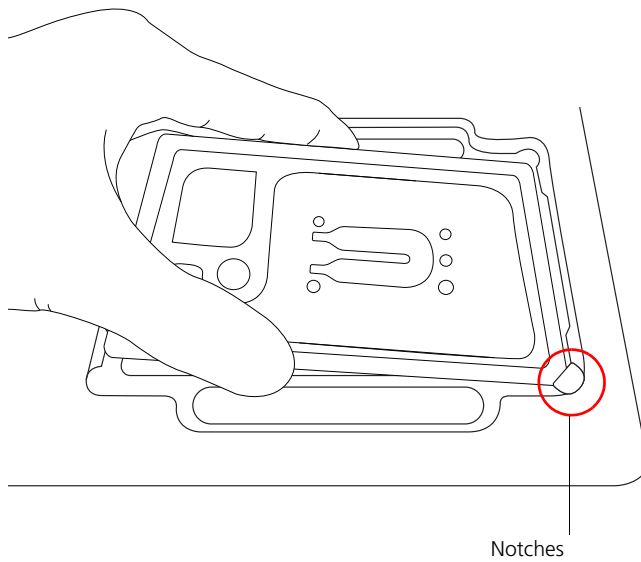
---

### Procedure

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

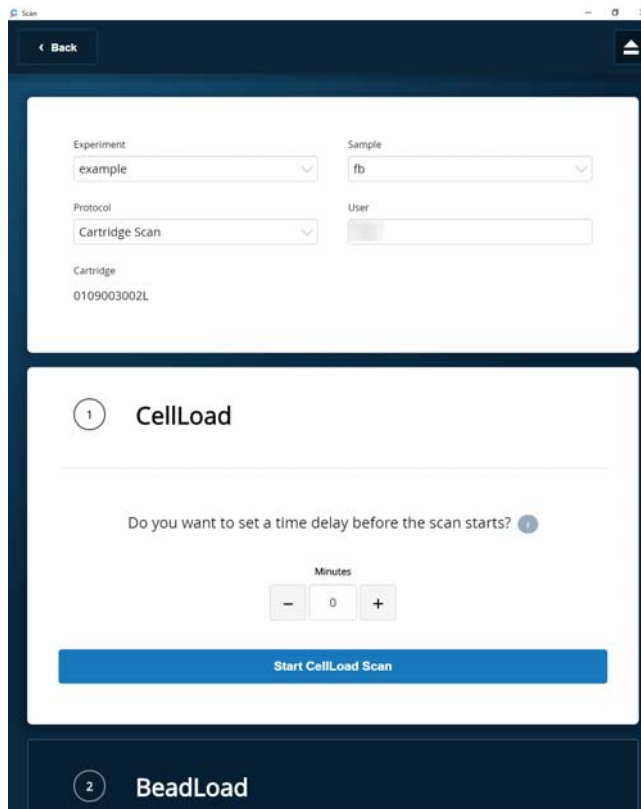
**Note:** If the tray is not ejected, tap the eject button in the upper right of the cartridge insertion screen.

2. Push the cartridge into the far end of the 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.
3. 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**:



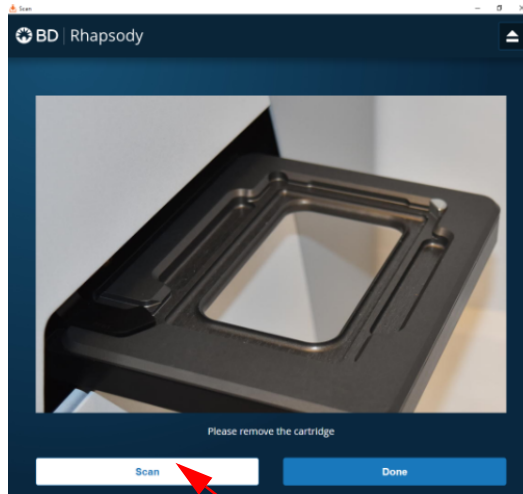
8. Proceed according to incubation location:
  - Express instrument (15 minute incubation completed): Proceed to step 9.
  - Incubation in scanner: Enter a 15 minute time delay to delay the start of the scan, and incubate the cartridge in the scanner by proceeding to step 9. During incubation in the scanner, prepare the Cell Capture Beads (Cat. No. 650000089). See [Preparing Cell Capture Beads \(page 93\)](#). After preparing the 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 Cell Capture Beads

---

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

### Preparing Cell Capture Beads

Use low retention pipette tips and LoBind Tubes when handling Cell Capture Beads.

1. Place the tube with Cell Capture Beads (Cat. No. 650000089) (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  $\mu$ L of cold Sample Buffer (Cat. No. 650000062) 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 Cell Capture Beads and imaging \(page 94\)](#).
-

## Loading Cell Capture Beads and imaging

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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  $\mu\text{L}$  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 Cell Capture Beads in cold Sample Buffer (Cat. No. 650000062) up and down to mix, and, using the BD Rhapsody P1200M pipette in **Bead Load** mode, immediately load the cartridge with 630  $\mu\text{L}$  of beads.
6. Let the beads settle in the cartridge on the tray at room temperature (15°C to 25°C) for 3 minutes.
7. Image the cartridge with the scanner (**Bead Load** step). For detailed instructions, see [Imaging cells in a cartridge \(page 89\)](#).
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  $\mu\text{L}$  of air or reagent. After aspiration, insert the tip into the cartridge, and then press the button once to dispense 700  $\mu\text{L}$  of air or liquid. After removing the pipette tip from the cartridge inlet, press the button once to dispense the remaining 20  $\mu\text{L}$  of air or liquid before ejecting the pipette tip.

10. Load the cartridge with 700  $\mu\text{L}$  of air using the BD Rhapsody P1200M pipette in **Wash** mode.
  11. Load the cartridge with 700  $\mu\text{L}$  of cold Sample Buffer (Cat. No. 650000062) 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 \(page 89\)](#).
- 

## Lysing cells and retrieving Cell Capture Beads

---

### Lysing the cells

Avoid bubbles.

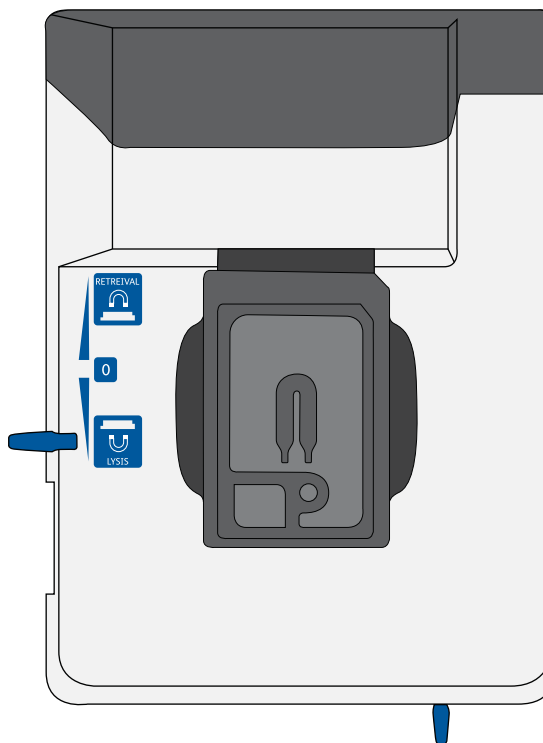
1. Add 75.0  $\mu\text{L}$  of 1 M DTT (Cat. No. 650000063) to one bottle of 15 mL Lysis Buffer (Cat. No. 650000064), and then check the **Add DTT** box on the Lysis Buffer label.

**Use the Lysis Buffer with DTT  $\leq$ 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.



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

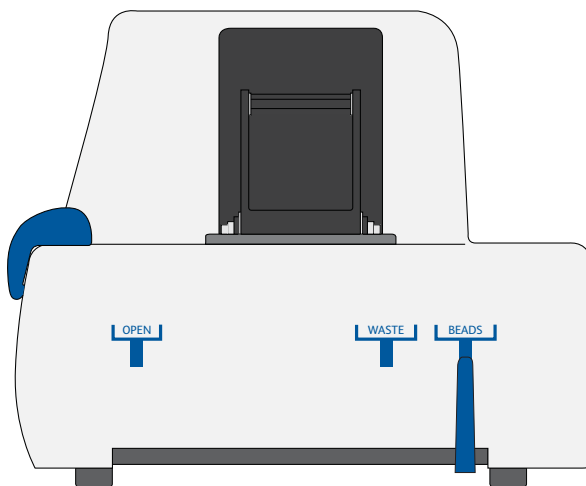


5. Change the mode on the BD Rhapsody P1200M pipette to **Lysis**.
6. Load the cartridge with 550  $\mu\text{L}$  of Lysis Buffer with DTT using the BD Rhapsody P1200M pipette in **Lysis** mode.
7. Leave the cartridge at room temperature (15°C to 25°C) on the tray for 2 minutes.

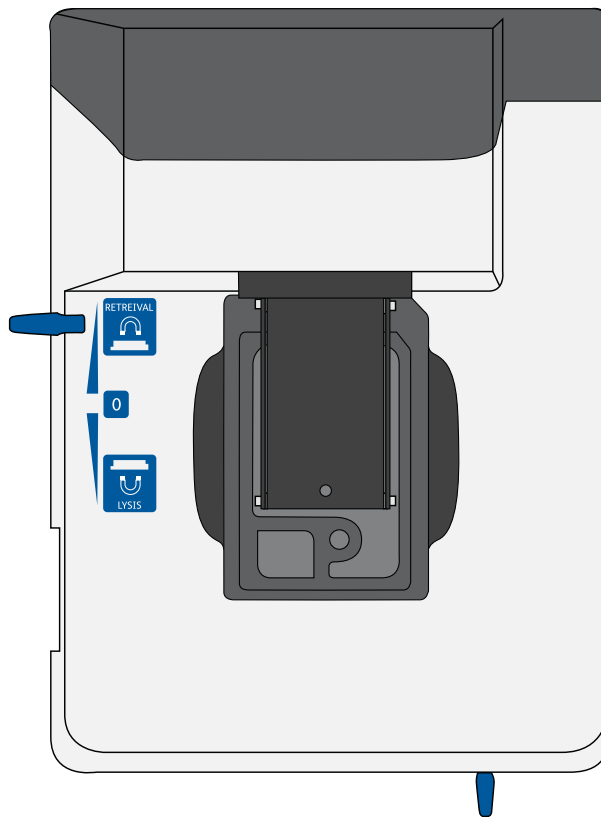
**Maintain recommended lysis time for best performance.**

**Retrieving the Cell Capture Beads from the cartridge**

1. Ensure that a 5 mL LoBind Tube (Eppendorf Cat. No. 0030108310) 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  $\mu\text{L}$  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 Cell Capture Beads (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.

**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 \(page 89\)](#).
13. Proceed immediately to [Performing reverse transcription on the Cell Capture Beads \(page 101\)](#) to process the beads and begin reverse transcription.
14. Appropriately dispose of the BD Rhapsody Cartridge according to biosafety level (BSL):

	<b>Biological hazard.</b>
---	---------------------------

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

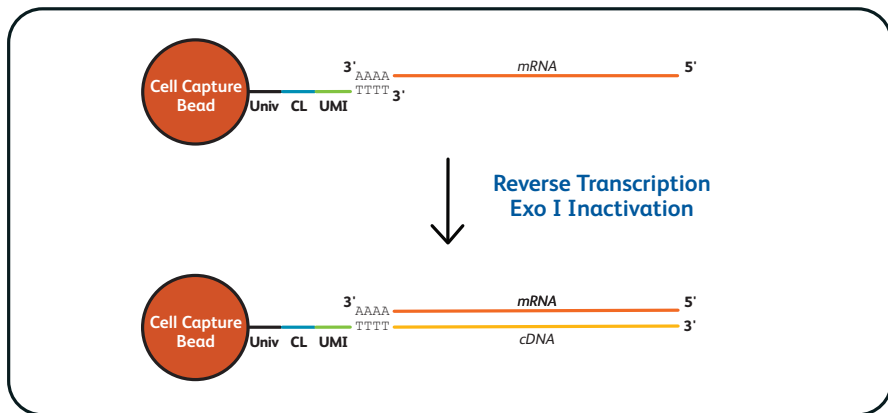
Dispose of waste using proper precautions and in accordance with local regulations. For more information, see [Waste \(page 138\)](#).

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% bleach or 70% ethyl alcohol. See the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide* (Doc ID 43084).
-

# Performing reverse transcription on the Cell Capture Beads

## Introduction

Prepare the reverse transcription mix, wash the 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  $\leq 30$  minutes after washing retrieved beads with Bead Wash Buffer.

## Before you begin

- Obtain the 5 mL LoBind Tube of retrieved beads. See [Retrieving the Cell Capture Beads from the cartridge \(page 97\)](#).
- Ensure that the SmartBlock™ Thermoblock 1.5 mL or equivalent is installed on the thermomixer and is set to 37°C and 20 minutes.

## Washing the Cell Capture Beads

Keep the Cell Capture Beads cold during washes.

Use low retention tips to handle Cell Capture Beads.

1. After the 1 minute incubation on the large magnet [see [Retrieving the Cell Capture Beads from the cartridge \(page 97\)](#)] and while leaving the 5 mL LoBind Tube on the large magnet, use a pipette to carefully remove all but ~1 mL of supernatant without disturbing the beads.
2. Remove the tube from the large magnet, resuspend the ~1 mL beads by gently pipetting the suspension up and down, and then transfer the bead suspension to a new 1.5 mL LoBind Tube.
3. If beads remain in the 5 mL LoBind Tube, pipet an additional 0.5 mL of Lysis Buffer with DTT into the tube, rinse the 5 mL tube, and transfer the suspension to the 1.5 mL LoBind Tube of beads.
4. Place the tube on the 1.5 mL tube magnet for  $\leq 2$  minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.

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

5. Remove the 1.5 mL LoBind Tube from the magnet, and then pipet 1.0 mL of cold Bead Wash Buffer (Cat. No. 650000065) into the tube. Gently mix the suspension by pipette only. Do not vortex.

6. Place the tube on the 1.5 mL tube magnet for  $\leq 2$  minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
7. Remove the 1.5 mL LoBind Tube from the magnet, and then pipet 1.0 mL of cold Bead Wash Buffer (Cat. No. 650000065) into the tube. Gently mix the suspension by pipette only, and place the tube on ice. Do not vortex.

**Start reverse transcription  $\leq 30$  minutes after washing retrieved Cell Capture Beads with Bead Wash Buffer.**

---

**Performing reverse transcription**

When working with Cell Capture Beads (beads), use only low retention tips and LoBind Tubes.

Limit preparation of mixes to  $\leq 20\%$  overage.

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 (Cat. No. 650000067)	40.0	48.0
dNTP (Cat. No. 650000077)	20.0	24.0
RT 0.1 M DTT (Cat. No. 650000068)	10.0	12.0
Bead RT/PCR Enhancer (Cat. No. 91-1082)	12.0	14.4
RNase Inhibitor (Cat. No. 650000078)	10.0	12.0
Reverse Transcriptase (Cat. No. 650000069)	10.0	12.0
Nuclease-Free Water (Cat. No. 650000076)	98.0	117.6
<b>Total</b>	<b>200.0</b>	<b>240.0</b>

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

4. Place the tube of washed beads [see [Washing the Cell Capture Beads \(page 102\)](#)] on the 1.5 mL tube magnet for  $\leq 2$  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  $\mu\text{L}$  of the cDNA mix to resuspend the beads. Gently mix the suspension by pipette only. Do not vortex.

**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.

**Shaking is critical for this incubation.**

**During reverse transcription incubation, view the image analysis to determine if the analysis metrics have passed. See [Reviewing the analysis metrics \(page 126\)](#).**

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 or heat block to 80°C.
- 

**Preparing the Exonuclease I mix**      When working with Cell Capture Beads, use only low retention tips and LoBind Tubes.

Limit preparation of mixes to  $\leq 20\%$  overage.

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 ( $\mu\text{L}$ )	1 library + 20% overage ( $\mu\text{L}$ )
10X Exonuclease I Buffer (Cat. No. 650000071)	20.0	24.0
Exonuclease I (Cat. No. 650000072)	10.0	12.0
Nuclease-Free Water (Cat. No. 650000076)	170.0	204.0
<b>Total</b>	<b>200.0</b>	<b>240.0</b>

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

---

**Treating the Cell  
Capture Beads with  
Exonuclease I**

1. Place the tube of beads with cDNA mix on the 1.5 mL tube magnet for  $\leq 2$  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  $\mu\text{L}$  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.
  4. If the thermomixer or heat block needs to preheat to a different temperature (80°C thermomixer or heat block), place the samples on ice until that temperature is reached.
  5. Immediately proceed to [Inactivating Exonuclease I](#).
- 

**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, or place the bead suspension in a heat block at 80°C for 20 minutes.
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 ( $\leq 1$  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  $\mu$ L of cold Bead Resuspension Buffer (Cat. No. 650000066) to gently resuspend the beads. Do not vortex.

**Stopping point:** The Exonuclease I-treated beads can be stored at 2°C to 8°C for  $\leq$ 3 months.

6. Proceed to library preparation. See the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524) to find the appropriate protocol to follow.
-

# 6

## Troubleshooting

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- Scanning troubleshooting (page 110)
- Cartridge loading troubleshooting (page 112)
- BD Rhapsody Scanner software messages (page 116)

## Scanning troubleshooting

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**Introduction** This topic describes possible problems and recommended solutions for scanning issues.

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### Incorrect alignment

Possible causes	Recommended solutions
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

Possible causes	Recommended solutions
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

Possible causes	Recommended solutions
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.

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Possible causes	Recommended solutions
Dirty optics	Contact BD Biosciences technical support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> or 1.877.232.8995.

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## Cartridge loading troubleshooting

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### Introduction

This topic describes possible problems and recommended solutions for BD Rhapsody™ Cartridge issues. These issues arise during image analysis. Also see [BD Rhapsody™ Scanner metrics \(page 121\)](#).

---

### Number of cells captured too high or cell doublet rate too high (out of range)

Possible causes	Recommended solutions
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 the BD Rhapsody™ Single-Cell Analysis system (page 67)</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>

---

**Number of cells captured lower than anticipated**

Possible causes	Recommended solutions
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	Cell capture efficiency is reduced in the BD Rhapsody Cartridge if the cell diameter is >20 µm.

**Bead loading density out of range**

Possible causes	Recommended solutions
Insufficient number of 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 (Cat. No. 650000062).</li> <li>• Use the <b>Bead 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 Cell Capture Beads**

Possible causes	Recommended solutions
Pipette mode incorrect	Ensure that the correct pipette mode is used for the step.
Pipette step missed	Review protocol, and repeat, if necessary.

**Cell retention out of range, indicating cell loss during cartridge workflow**

Possible causes	Recommended solutions
Pipette mode incorrect	Ensure that the correct pipette mode is used for the step.
Cell viability low	Use cells of high viability.

**Bead retrieval efficiency out of range, indicating poor retrieval**

Possible causes	Recommended solutions
Retrieval (top) magnet not positioned properly	Check magnet position.
Pipette mode incorrect	Ensure that the correct pipette mode is used for the step.

**Image analysis does not start**

Possible causes	Recommended solutions
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. Tap <b>Analyze</b> to start the analysis. You can analyze multiple scan steps at the same time.</li> </ol>

**Dropped the cartridge or hit it against object**

Possible causes	Recommended solutions
Various	<ul style="list-style-type: none"> <li>• If the cartridge was dropped, BD recommends 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>

**Air bubble in cartridge**

Possible causes	Recommended solutions
Air bubble present in pipette tip while dispensing buffer	<p>Confirm that there is an air bubble in the cartridge by examining an image of the cartridge.</p> <p>Ensure that the pipette tip contains only buffer and no air bubble is trapped in the cartridge at the end of aspiration.</p>
Re-used pipette tip	Use a new pipette tip at every pipetting step.

**Image analysis fails**

Contact BD Biosciences technical support at [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) or 1.877.232.8995.

**Installation or mechanical issues**

See the *BD™ Rhapsody Single-Cell Analysis System Installation and Maintenance Guide* (Doc ID: 43084).

## BD Rhapsody Scanner software messages

### Introduction

This topic 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) or 1.877.232.8995.

### Error messages

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

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>

Error message (continued)	Possible causes	Recommended solutions
“Available disk space is extremely low.”	Low disk space	Contact BD tech support <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a> or 1.877.232.8995.
“Failed to initialize...”	Various	Contact BD tech support <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a> or 1.877.232.8995.
“Requested move not safe.”	Various	Contact BD tech support <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a> or 1.877.232.8995.
“Unknown cartridge barcode.”	Unsupported barcode scanned	Contact BD tech support <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a> or 1.877.232.8995.
“Failed to read file.”	Various	Contact BD tech support <a href="mailto:scomix@bdscmix.bd.com">scomix@bdscmix.bd.com</a> or 1.877.232.8995.
“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 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. 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> or 1.877.232.8995.
“The protocol was not found.”	Protocol missing	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> or 1.877.232.8995.
“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> or 1.877.232.8995.
“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> or 1.877.232.8995.
“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> or 1.877.232.8995.
“Auto-exposure adjustment unsuccessful. Using nearest value.”	Algorithm calculation	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> or 1.877.232.8995.

Warning message (continued)	Possible causes	Recommended solutions
“Autofocus did not find perfect focus. Using nearest value.”	<ul style="list-style-type: none"> <li>● Bubble in cartridge</li> <li>● Calibration needs update</li> </ul>	Contact BD tech support <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> or 1.877.232.8995.
“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> or 1.877.232.8995.



Warning message (continued)	Possible causes	Recommended solutions
“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"> <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> or 1.877.232.8995.
“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 <a href="mailto:scomix@bdscomix.bd.com">scomix@bdscomix.bd.com</a> or 1.877.232.8995.

# A

## **BD Rhapsody™ Scanner metrics**

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- Image analysis metrics (page 122)
- Reviewing the analysis metrics (page 126)

## Image analysis metrics

**Understanding analysis metrics** The chart lists the steps and metrics measured during image analysis of the hemocytometer or BD Rhapsody™ Cartridge.

Step	Metric	Definition	Use	Passing threshold
Hemocytometer	Total cell concentration (cell/ $\mu$ L)	Number of viable (Calcein AM-positive) and non-viable (DRAQ7-positive) cells/ $\mu$ L	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>	—

Step (continued)	Metric	Definition	Use	Passing threshold
Cell Load	Cell multiplet rate at Cell Load <sup>d</sup> (%)	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 $\geq 1$ 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 Cell Capture Bead at cell lysis. <sup>c</sup>	—
Bead Wash	Cell multiplet rate <sup>d</sup> (%)	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

Step (continued)	Metric	Definition	Use	Passing threshold
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%

- 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.
- b. Cell capture efficiency at Cell Load (%) =  $\frac{\text{Number of viable cells captured in well at Cell Load}}{\text{Total number of viable cells imaged}} * 100$ .
- 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.
- 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 \(page 67\)](#). 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.

**Viewing image  
analysis metrics**

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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.

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## 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 \(page 122\)](#). 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 \(page 29\)](#).

- (1) **Analysis metrics** ([page 128](#))
- (2) **Cell count** ([page 129](#))
- (3) **Sample result** ([page 129](#))



## (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

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# B

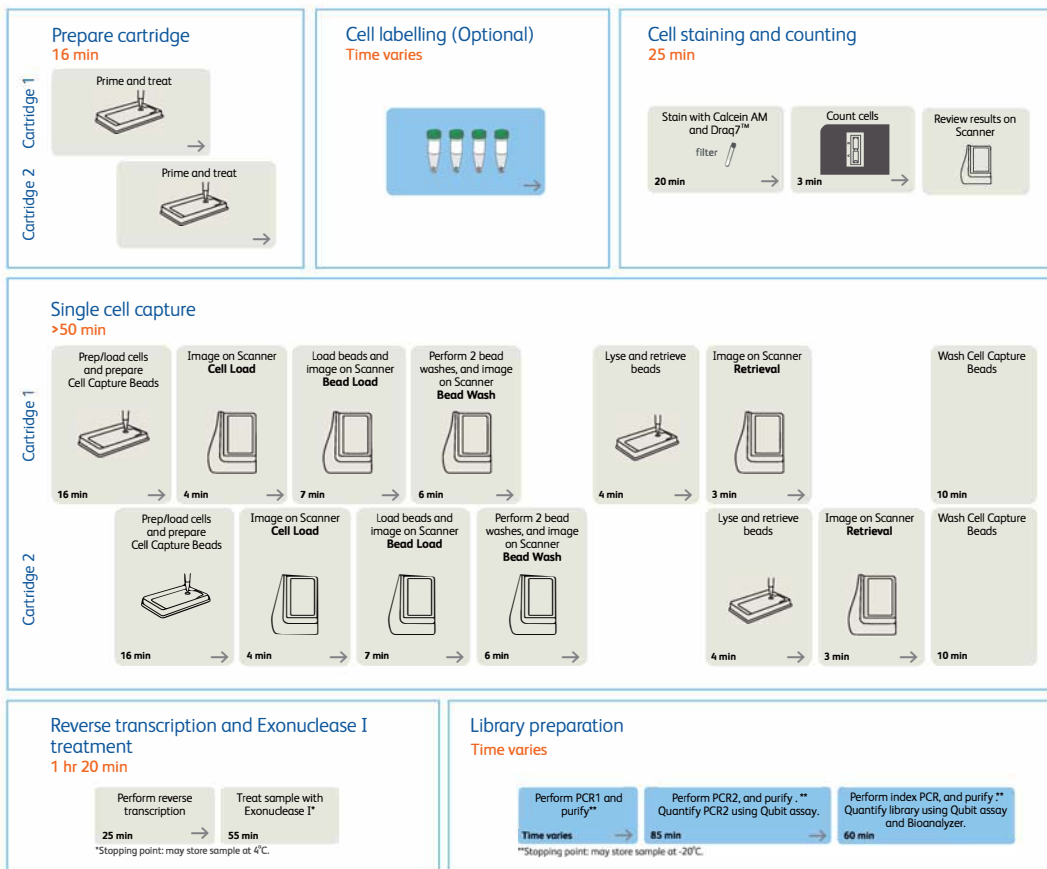
## **Workflow with two BD Rhapsody™ Cartridges**

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- [Workflow with two cartridges \(page 132\)](#)
- [Best practices with a two-cartridge workflow \(page 133\)](#)

# Workflow with two cartridges

Staggered boxes indicate staggering the start of like steps.



## Best practices with a two-cartridge workflow

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### 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.

To perform the workflow, follow the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

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# C

## **Updating the BD Rhapsody™ Scanner software**

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- Introduction (page 136)



## Introduction

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BD Biosciences recommends 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 (Part Number: 23-20709-01)*.

# D

## Safety

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- General safety and limitations (page 138)
- Chemical safety (page 138)
- Physical safety (page 140)
- Instrument waste disposal (page 140)

## General safety and limitations

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For instrument safety, see the *BD Rhapsody™ Express Instrument and BD Rhapsody™ Scanner Safety and Limitations Guide* (Doc ID: 42061).

Genomics technical publications are available for download from the BD Genomics Resource Library at [bd.com/genomics-resources](https://bd.com/genomics-resources).

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## 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.
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### 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 microparticles (&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 microparticles (&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™ Express Instrument and BD Rhapsody™ Scanner Safety and Limitations Guide* (Doc ID: 42061).

Genomics technical publications are available for download from the BD Genomics Resource Library at [bd.com/genomics-resources](https://bd.com/genomics-resources).

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## Instrument waste disposal

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### Disposal of the instrument

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™ Express instrument or the BD Rhapsody Scanner. For more information, see [Instrument technical support \(page 13\)](#).

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# Glossary

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## B

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**BD Rhapsody™ Express instrument** Mechanical station used for loading Cell Capture Beads and cells into the BD Rhapsody™ Cartridge.

**BD Rhapsody™ Scanner** Instrument used for counting cells on a hemocytometer and counting viable cells in the cartridge workflow.

**BD Rhapsody™ Single-Cell Analysis system** The system includes:

- BD Rhapsody Express instrument
- BD Rhapsody™ P1200M pipette
- BD Rhapsody™ P5000M pipette
- BD Rhapsody Scanner
- Hemocytometer Adapter
- Bioinformatics software and sequencing analysis

## L

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**load** To add a reagent to the BD Rhapsody Cartridge.

## P

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**protocol** The protocol files for the BD Rhapsody Scanner describe all instrument settings for a cartridge scan and the order in which the scans occur.

## S

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**scan** A series of images taken of the Hemocytometer Adapter or BD Rhapsody Cartridge by the BD Rhapsody Scanner at multiple predetermined locations.

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