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

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Regulatory information

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

Patents

The BD Rhapsody™ Targeted Reagent Kit is covered by one or more of the following U.S. patents: 8835358, 9315857, 9290808, 9290809, 9290809, 9567646, and 9598736.

Limited use license for genome amplification

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History

Revision	Date	Changes made
Doc ID: 214063 Rev. 1.0	07/2018	Initial release

Contents

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Introduction

About this guide

Introduction

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

To use the BD Rhapsody™ Scanner for image analysis of BD Rhapsody™ Cartridge loading, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID: 214062). The scanner is not required in the BD Rhapsody™ Express workflow.

Genomics technical publications are available for download from the BD Genomics Resource Library at bd.com/genomics-resources.

Intended use

Intended use

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

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

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

For more information on the purpose of the BD Rhapsody Express instrument, see the BD Rhapsody Express instrument overview (page 20).

Restrictions

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

BD denies any responsibility for damage caused by the following:

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

Disclaimer

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

Safety symbols

Introduction

This topic describes the safety symbols used in this guide.

For safety and limitations, see the *BD Rhapsody™ Express instrument Safety and Limitations Guide* (Doc ID: 76918).

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.

Other symbols

Symbol	Meaning
	Part number
	Lot number
	Storage temperature range
	Expiration date
	Consult instructions for use

Safety data sheets

Introduction

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

Obtaining SDSs

Before handling chemicals, read and understand the SDSs. To obtain SDSs for chemicals ordered from BD Biosciences, go to regdocs.bd.com, or contact BD Biosciences technical support at researchapplications@bd.com.

Instrument technical support

Introduction

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

Contacting technical support

If technical assistance is required, contact BD Biosciences technical support at researchapplications@bd.com or 1.877.232.8995, prompt 2, 2. You can contact technical support in Europe at 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
- Details of recent system performance

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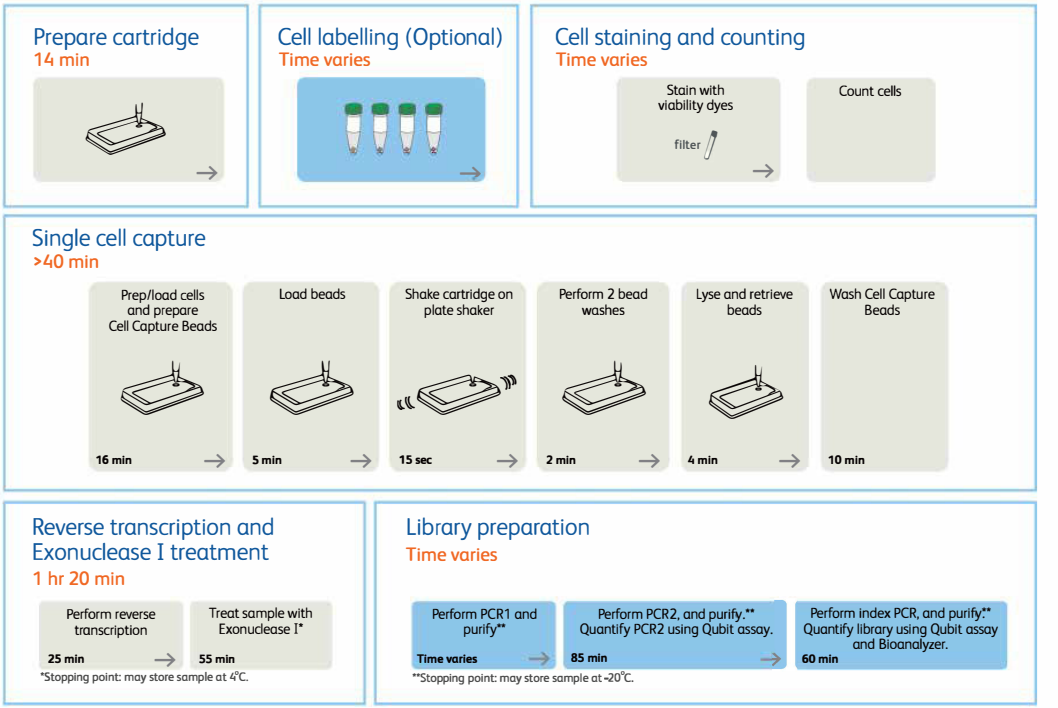
Getting Started

BD Rhapsody™ Express Targeted library workflow



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

Note: When using two cartridges, see Workflow with two BD Rhapsody™ Cartridges (page 119).

Site requirements

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 Single-Cell Analysis System Site Preparation Guide* (Doc ID: 75750).

For installation of the BD Rhapsody™ Express instrument, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express instrument* (Doc ID: 76955).

Genomics technical publications are available for download from the BD Genomics Resource Library at bd.com/genomics-resources.

Plate shaker settings

Settings

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

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

Use other plate shakers at your own risk.

Thermomixer settings

Settings

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

Thermal cycler setup

Recommendations

- Use a properly calibrated thermal cycler for 0.2 mL tubes with a maximum reaction volume of 50 µL.
- Use a heated lid set to ≥95°C.
- Do not use fast cycling mode.
- For specific instrument operation, follow the instructions provided by the manufacturer.

Pipette Settings

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**

BD Rhapsody Express instrument overview

Introduction

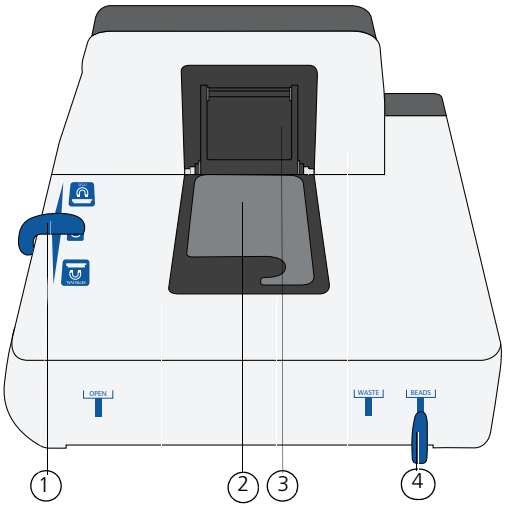
The BD Rhapsody Cartridge requires the use of the BD Rhapsody Express instrument. The station is used to load reagents, cells, and Cell Capture Beads into the cartridge for bead capture and retrieval of single cell mRNA.

Safety

For safety and limitations of the BD Rhapsody Express instrument, see the *BD Rhapsody™ Express instrument Safety and Limitations Guide* (Doc ID: 76918).

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™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument* (Doc ID: 76955).



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

Best practices

Good laboratory practices

- Calibrate and service pipettes every 12 months to ensure accurate sample volume transfer at each step. To clean and calibrate the pipettes, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument* (Doc ID: 76955).
- 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 to minimize bead loss.
- Wear disposable gloves, and change them frequently.
- Never reuse tips or tubes.

	• Keep tip boxes, reagent containers, and sample tubes closed when not in use. • Always maintain a clean laboratory bench, and if necessary, wipe work surface with a solution of 10% (v/v) bleach.
Cell handling and cell counting	See Best practices for cell handling and cell counting (page 60).
Sterility	• Clean cell culture surfaces in the laminar flow hood with 70% (v/v) ethyl alcohol, and appropriately sterilize the surfaces. • Use sterile serological pipettes to aseptically transfer media and cells. • Place flasks in a cell culture hood one at a time to prevent cross-cell contamination.
Cell Capture Beads	• Always keep Cell Capture Beads on ice when not in 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 BD Rhapsody Express instrument contains strong magnets. Keep metal objects away from the station. • Wipe the BD Rhapsody Express instrument with 70% ethyl alcohol wipes after each use.

BD Rhapsody Cartridge

- Avoid pipetting bubbles into the cartridge. Before adding fluid to the cartridge, ensure that the pipette tip does not contain air.
- To ensure an air-tight seal with the 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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Product information

Required kit and storage conditions

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 additional kit is required:

- BD™ Single-Cell Multiplexing Kit (Cat. No. 633781)

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

- BD™ AbSeq Ab-Oligos (antibody-oligonucleotides)
- BD™ Stain Buffer (FBS)
- (Optional) 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 ≤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 µL	
	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

Box	Component	Cap color	Quantity	Volume per unit	Storage
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 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	

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 researchapplications@bd.com, and see BD Rhapsody™ Panel Supplement (page 36).

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 27).

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	
BD Rhapsody™ Training Cells (Cat. No. 633741) • Jurkat • Ramos	• 2 vials, 1.0 mL each • 2 vials, 1.0 mL each	Liquid nitrogen

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 researchapplications@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[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	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[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

Panel	Component	Cap color	Quantity	Volume	Storage
BD Rhapsody Custom Panel 100–199 genes (Outside U.S.: Cat. No. 633778)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	
BD Rhapsody Custom Panel 200–299 genes (Outside U.S.: Cat. No. 633779)	BD Rhapsody™ 10X PCR1 Custom ID: xxxxxx[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	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[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	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[a]	Blue	1 vial	1.4 mL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Custom ID: xxxxxx[a]	Blue	1 vial	350 µL[b]	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

a. Each design has a unique identifier that is provided with your BD Rhapsody Custom Panel primer design.

b. 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

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 researchapplications@bd.com.

Up to two BD Rhapsody Panel Supplements can be added per reaction, provided that the total number of primers added is ≤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[a]	Blue	1 vial	120 µL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx[a]	Blue	1 vial	30 µL[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[a]	Blue	1 vial	120 µL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx[a]	Blue	1 vial	30 µL[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[a]	Blue	1 vial	120 µL[b]	–25°C to –15°C
	BD Rhapsody™ 10X PCR2 Supp. ID: xxxxxx[a]	Blue	1 vial	30 µL[b]	
	IDTE pH 8.0	Blue	1 vial	2.0 mL	

a. Each design has a unique identifier that is provided with your BD Rhapsody Panel Supplement primer design.
b. 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.

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

Reagents

Required reagent for BD Rhapsody Cartridge workflow

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

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

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

Required reagents for cell preparation and staining

Reagent	Supplier	Catalog no.
Phosphate-buffered saline (calcium- and magnesium-free)	Major supplier	—
70% ethyl alcohol or 70% isopropyl alcohol[a]	Major supplier	—

a. To clean the BD Rhapsody™ Express instrument, see the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument* (Doc ID: 76955). Instead of 70% (v/v) 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

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

Material	Supplier	Catalog no.
Human BD Fc Block™[a]	BD Biosciences	564220

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

Suggested reagents for cell counting

Reagent	Supplier	Catalog no.
Calcein AM cell-permeant dye[a]	Thermo Fisher Scientific	C1430
Dimethyl sulfoxide (DMSO)	Major supplier	—
Propidium Iodide, 1 mg/mL in Water[a]	Thermo Fisher Scientific	P3566
Trypan Blue Stain, 0.4%	Thermo Fisher Scientific	15250061

a. Required for counting by viability stain and fluorescence microscope. See Counting cells by fluorescence stains and fluorescence microscopy (page 62). Protect Calcein AM and Propidium Iodide from light. Avoid multiple freeze-thaw cycles of Calcein AM. See manufacturer's storage recommendations.

Consumables

Required consumables

Consumable item	Supplies	Catalog no.
Falcon® Tube with Cell Strainer Cap	Corning	352235
Falcon tubes, 5 mL Round Bottom Polystyrene Test Tube[a]	Corning	352054
DNA LoBind Tubes, 1.5 mL[b]	Eppendorf	0030108051
DNA LoBind Tubes, 5 mL **Note:** These are the Bead Retrieval Tubes to be used with the BD Rhapsody Express instrument.	Eppendorf	0030108310
Low retention filtered pipette tips, 10 µL[b]	Major supplier	—
Low retention filtered pipette tips, 200 µL[b]	Major supplier	—
Low retention filtered pipette tips, 1,000 µL[b]	Major supplier	—

Consumable item (continued)	Supplies	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[c]	Thermo Fisher Scientific	4900 or 4890
0.2 mL PCR 12-strip tubes or 0.2 mL PCR tubes[a]	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.

Suggested consumables

Consumable item	Supplier	Catalog no.
Improved Neubauer Hemocytometer[a]	INCYTO	DHC-N01-5
Cell Counting Slides for TC20™ Cell Counter	Bio-Rad	1450011
BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube–Sodium Heparin[b]	BD Biosciences	362753

a. For cell counting under a microscope.
b. 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 item	Components
BD Rhapsody Express Single-Cell Analysis system (Cat. No. 633707)	• BD Rhapsody Express instrument (Cat. No. 633702)[a] • BD Rhapsody P1200M pipette (Cat. No. 633704)[a] • BD Rhapsody P5000M pipette (Cat. No. 633705)[a]

a. Item can be ordered separately.

Equipment item	Supplier	Catalog no.
6-Tube Magnetic Separation Rack for 1.5 mL tubes[a]	New England BioLabs	S1506S
Large magnetic separation stand	V&P Scientific, Inc.	VP 772FB-1
Clear acrylic cylinder adapter for 15 mL tube magnet[b]	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

Equipment item (continued)	Supplier	Catalog no.
Eppendorf ThermoMixer® C[c]	Eppendorf	5382000023
SmartBlock™ Thermoblock 1.5 mL to fit on the ThermoMixer® C[c]	Eppendorf	5360000038
A plate shaker for cartridge workflow: SmartBlock™ plates (for ThermoMixer C) OR	Eppendorf	5363000039
Eppendorf MixMate® OR	Eppendorf	022674200
MicroPlate Genie™	Scientific Industries, Inc.	SI-0400
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
• 2100 Bioanalyzer OR • 4200 TapeStation Instrument	Agilent Technologies	• G2940CA • G2991AA
Thermal cycler with heated lid	Major supplier	—

Equipment item (continued)	Supplier	Catalog no.
Water bath OR incubator at 37°C	Major supplier	—
Laminar flow hood	Major supplier	—
Digital timer[a]	Major supplier	—
Pipettes (P10, P20, P200, P1000)[a]	Major supplier	—
Multi-channel pipette, 2–20 µL OR 20–200 µL	Major supplier	—
Microcentrifuge for 1.5–2.0 mL tubes[a]	Major supplier	—
Microcentrifuge for 0.2 mL tubes	Major supplier	—
Centrifuge and rotor with adapters for 5 mL Falcon tubes and for 15 mL tubes	Major supplier	—
Vortexer[a]	Major supplier	—
Pipet-Aid	Major supplier	—

a. Provide material in both pre- and post-amplification workspaces.
b. Holds 5 mL LoBind Tube in magnet.
c. Required for reverse transcription and Exonuclease I treatment.

Suggested equipment

Equipment item	Supplier	Catalog no.
TC20™ Automated Cell Counter	Bio-Rad	1450102
Fluorescence widefield microscope capable of the following excitation/emission: • Channel: 494 nm/517 nm • Channel: 533 nm/617 nm	Major supplier	—
Brightfield microscope	Major supplier	—
Phase-contrast microscope	Major supplier	—
8-Channel Screw Cap Tube Capper **Note:** Optional for antibody-oligonucleotide labelling.	Thermo Fisher Scientific	4105MAT

Software

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).

BD™ Data View

BD Data View is a software tool for visualization and exploratory analysis of output files generated following bioinformatics analysis, including output files from the BD Rhapsody Analysis pipeline. The software is included with the purchase of the BD Rhapsody 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).

4

Preparing the BD Rhapsody™ Cartridge

Priming the BD Rhapsody Cartridge

Before you begin

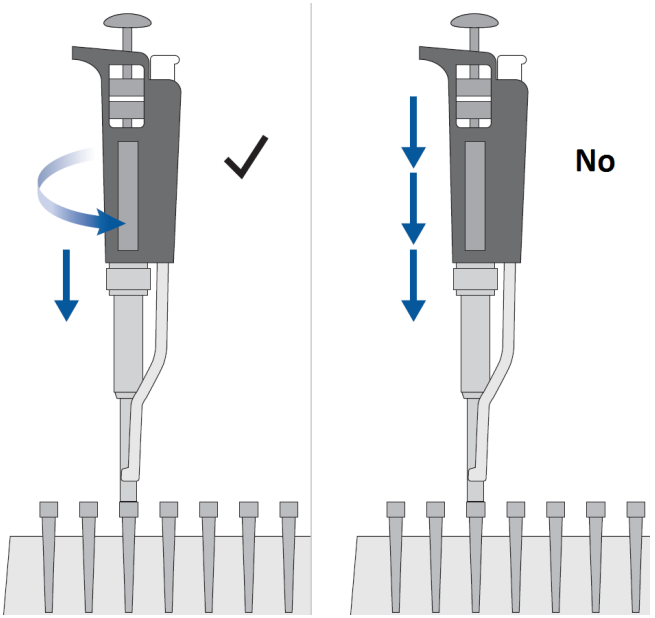
After opening the cartridge packet, ensure that you prepare the cartridge ≤1 day before use. If you leave the cartridge at 2°C to 8°C, equilibrate to room temperature (15°C to 25°C) for 5 minutes.

If cell preparation takes ≥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°C to 25°C) ≥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)
- Review pipette settings and operation. See Pipette Settings (page 19).
- For the use of two cartridges, see Workflow with two BD Rhapsody™ Cartridges (page 119).

Procedure

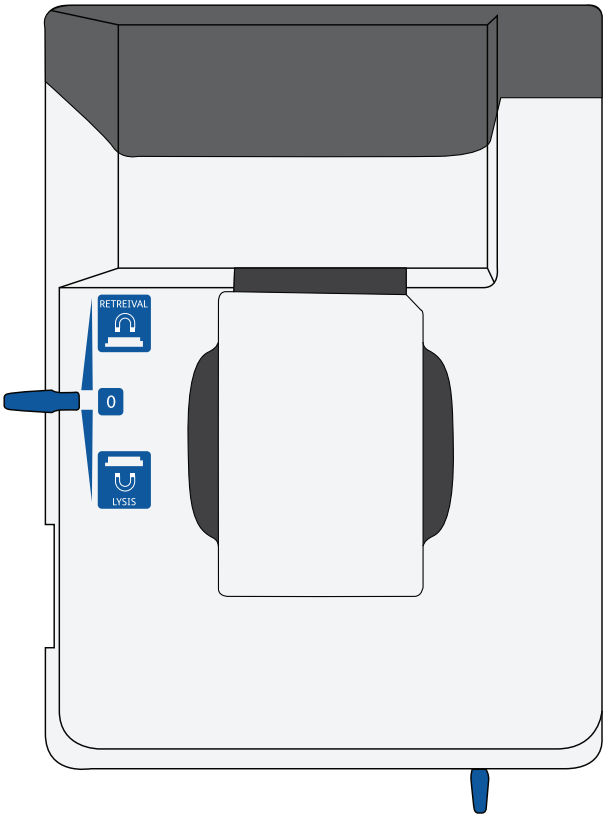
To ensure an air-tight seal with the BD Rhapsody™ P1200M or P5000M pipette, hold the pipette with one hand, and slightly twist the pipette to firmly seat a pipette tip on the pipette shaft:



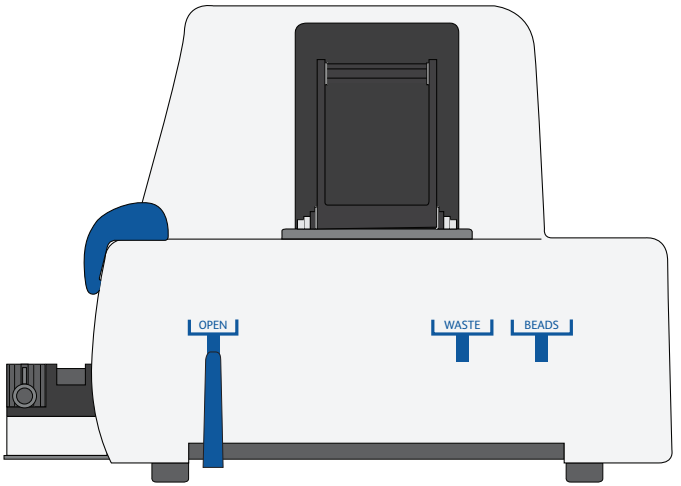
Avoid introducing bubbles while pipetting into the BD Rhapsody Cartridge.

Change pipette tips before every pipetting step.

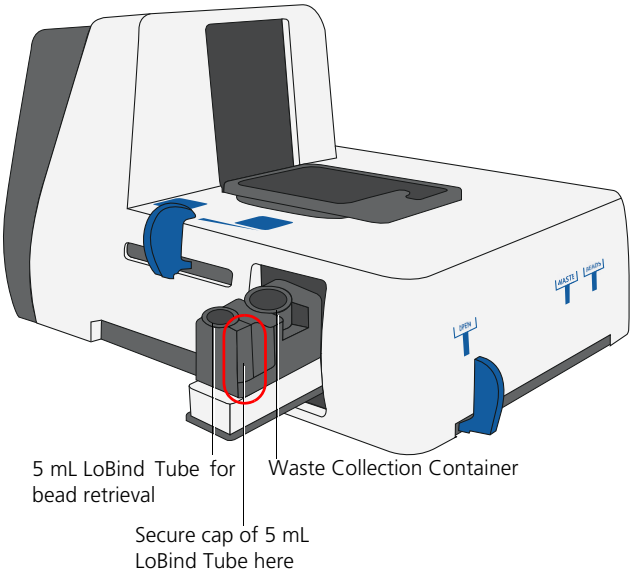
1. Move the left slider to the middle (**0**) position on the BD Rhapsody™ Express instrument. The Retrieval (top) magnet and Lysis (bottom) magnets are away from the cartridge tray:



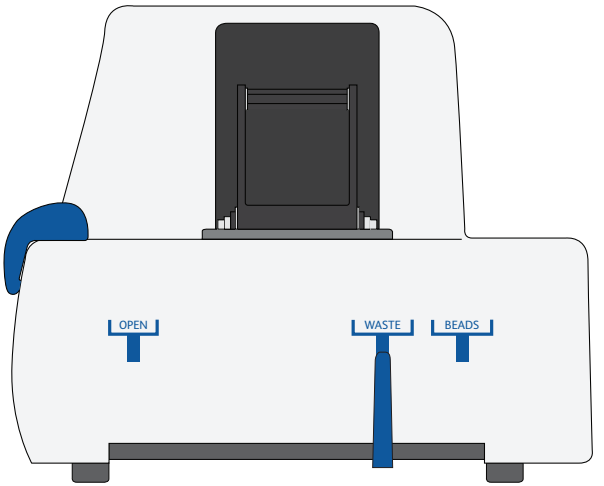
2. Move the front slider to **OPEN**:



3. Remove the cap of a Waste Collection Container (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:

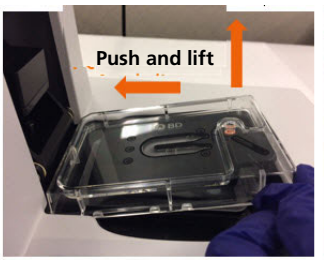


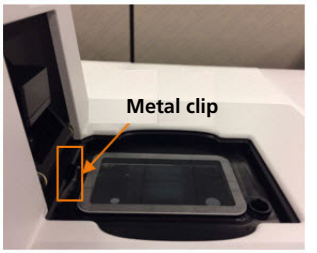
4. Move the front slider to **WASTE**:



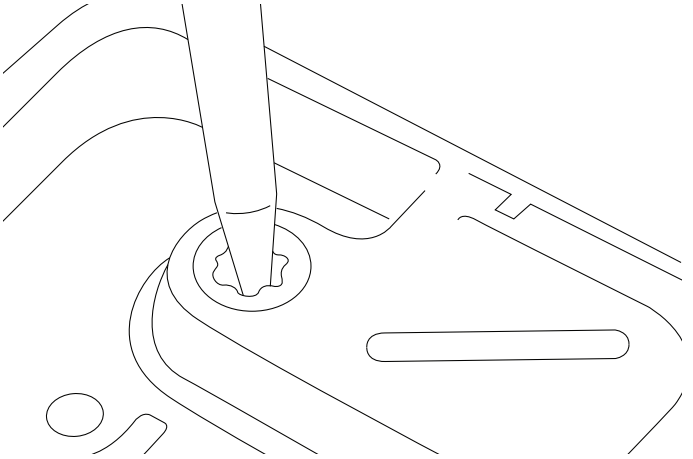
5. Push the cartridge into the far end of the tray to match the cartridge and tray notches. Lay the cartridge flat, and release it. Ensure that the cartridge is flat in the tray and the barcode faces out.

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





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



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

6. Load the cartridge with 700 µL of 100% (absolute) ethyl alcohol using the P1200M pipette in **Prime/Treat** mode.
7. Load the cartridge with 700 µL of air using the P1200M pipette in **Prime/Treat** mode.
8. Load the cartridge with 700 µL of Cartridge Wash Buffer 1 (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 µL of air using the P1200M pipette in **Prime/Treat** mode.
2. Load the cartridge with 700 µL 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 µL of air using the P1200M pipette in **Prime/Treat** mode.
5. Load the cartridge with 700 µL 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 ≤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. Proceed to the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524) to find the appropriate protocol for preparing cells.

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5

Counting cells

Best practices for cell handling and cell counting

Cell handling

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

Cell counting

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

- Filter cells to remove clumps and debris to ensure accurate cell counting. Debris in suspensions of small cells can lead to overestimated cell counts. Visually check filtered cell suspensions for debris.
- Avoid pipetting low volumes of cells (<2 µL).
- Concentrate cells as needed, and then recount them using one of the recommended methods in Cell counting methods (page 62).
- Do not rely on FACS-based counts, because cell concentration might be overestimated by this method. Always re-count cells after FACS using one of the recommended methods in Cell counting methods (page 62).

- If you are counting cells for the first time or if the cells of interest are of varying sizes, use at least two different counting methods. Counting by fluorescence is particularly appropriate with peripheral blood mononuclear cells (PBMCs), samples with debris, or samples with a significant fraction of non-nucleated cells.
- Treat cells with viability stain(s), and then count them by using one of the recommended methods. See Cell counting methods (page 62). The most accurate cell counting is achieved in this order: manual counting with fluorescence > automated counting with fluorescence > automated counting with Trypan Blue Stain and brightfield > manual counting with Trypan Blue Stain and brightfield.
- Be aware and adjust for limitations in cell counting methods, because there can be variations in cell counts. When using automated cell counters, be sure to use the appropriate settings validated for the cells of interest; for example, cell size, brightness, and circularity. When manually counting cells under the microscope, cell counts might vary due to brightness and focus.
- For accurate cells counts, take three separate aliquots from completely suspended cells, and count each aliquot once. Use the median cell concentration.
- Count cells that have been diluted to the recommended range for the cell counting method. See Assessing cell concentration (page 67). If the concentration is outside that range, dilute or concentrate the cells, accordingly, and recount them.
- Calculate the cell concentration for the total number of cells, not just for live cells. Use the total number of cells to determine the volume of stock cell suspension loaded into the cartridge.

Cell counting methods

Introduction

Count cells by one of three methods:

- Counting cells by fluorescence stains and fluorescence microscopy (page 62)
- Counting cells by Trypan Blue staining and brightfield microscopy (page 65)
- Counting cells by automated cell counting (page 66)

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).

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

Counting cells by fluorescence stains and fluorescence microscopy

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

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

1. If the cells are not already resuspended in 620 µL of cold Sample Buffer (Cat. No. 650000062), centrifuge the cell suspension at 400 × g for 5 minutes, aspirate the supernatant,

and leave ~20 µL of the residual supernatant. Add 620 µL total volume of cold Sample Buffer, and then proceed with cell staining in step 2.

2. Add 3.1 µL of 2 mM Calcein AM and 4.1 µL of 1 mg/mL Propidium Iodide in Water to the 620 µL volume of cell suspension.
3. Gently pipet the suspension up and down to mix well.
4. Incubate the suspension in the dark in a water bath, incubator, or heat block at 37°C for 5 minutes.
5. Pass the cells through a Falcon® Tube with Cell Strainer Cap (Thermo Fisher Scientific Cat. No. 352235).

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

   Count cells immediately.

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

- >160 cells/1 × 1 mm^2 square, dilute the cell suspension in cold Sample Buffer (Cat. No. 650000062), and recount the cells.

8. Calculate the concentration of the stock cell suspension and the cell viability:

   $$\text{Viable cells}/\mu L = \text{No. of live cells} \div \text{no. squares counted} \times 10$$

   $$\text{Total no. of cells}/\mu L = (\text{No. live cells} + \text{no. dead cells}) \div (\text{no. squares counted}) \times 10$$

   $$\text{Percent viable cells} = (\text{Viable cells}/\mu L) \div (\text{total cells}/\mu L) \times 100$$

9. Record the total cell concentration (cells/µL), live cell concentration (cells/µL), and percent viability.
10. Repeat steps 1–9 for each new sample if multiplexing samples. Assess total cell concentration, live cell concentration, and percent viability from three independent aliquots of your sample. Identify the aliquot with the median total cell concentration and use values (total cell concentration, live cell concentration, and viability) from this replicate.
11. If using a disposable hemocytometer, dispose of it according to local safety regulations. If using a non-disposable hemocytometer, clean it according to local safety regulations.
12. Proceed immediately to Assessing cell concentration (page 67).

Counting cells by Trypan Blue staining and brightfield microscopy

1. Pass the cells through a Falcon Tube with Cell Strainer Cap (Thermo Fisher Scientific Cat. No. 352235).

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

2. Gently mix cells well by pipette, and then pipet 10 µL from the center of the suspension into a new 1.5mL LoBind Tube.

3. Add 10 µL of 0.4%Trypan Blue Stain (Thermo Fisher Scientific Cat. No. 15250061).

4. Gently mix the cells by pipette, and then pipet 10 µL from the center of the stained cell suspension into one chamber of the INCYTO disposable hemocytometer or a non-disposable hemocytometer with coverslip.

5. If counting multiple aliquots of sample, use a new pipette tip each time to repeat steps 2–3 two more times for a total of three tubes of cells with Trypan Blue Stain.

6. Count the unstained (live) and stained (dead) cells in the four corner 1 × 1 mm^2 squares of the hemocytometer (To view the grid of the Neubauer Improved hemocytometer, see incyto.com.) If the live + dead cell count is:

   - 100–640 cells total in four 1 × 1 mm^2 squares, proceed to step 7.
   - <100 total in four 1 × 1 mm^2 squares: Count all nine 1 × 1 mm^2 squares. If the cell count is <100 after counting nine squares, count cells in another hemocytometer or centrifuge the cell sample, and recount the concentrated sample.
   - >160 cells/1 × 1 mm^2 square, dilute the cell suspension in cold Sample Buffer (Cat. No. 650000062), and recount the cells.

7. Calculate the concentration of the stock cell suspension and the cell viability:

   $$\text{Viable cells/µL} = \text{No. live cells} \div (\text{no. squares counted}) \times 2 \times 10$$

   $$\text{Total cells/µL} = (\text{No. live cells} + \text{no. dead cells}) \div (\text{no. squares counted}) \times 2 \times 10$$

   $$\text{Percent viable cells} = (\text{Viable cells/µL}) \div (\text{Total cells/µL}) \times 100$$

8. Record the total cell concentration (cells/µL), live cell concentration (cells/µL), and percent viability.
9. Repeat steps 1–8 for each sample if multiplexing samples. Assess total cell concentration, live cell concentration, and percent viability from three independent aliquots of your sample. Identify the aliquot with the median total cell concentration, and use values (total cell concentration, live cell concentration, and viability) from this replicate.
10. If using a disposable hemocytometer, dispose of it according to local safety regulations. If using a non-disposable hemocytometer, clean it according to local safety regulations.
11. Proceed immediately to Assessing cell concentration (page 67).

Counting cells by automated cell counting

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

Assessing cell concentration

Procedure

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

Cell concentration ranges of sample for precision counting

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

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

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

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

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

2. Proceed as follows:
   - If the cell concentration of every sample is within the range of the counting method, proceed immediately to single cell capture. If the cell concentration is below range, centrifuge the cell sample, and recount the concentrated sample.
   - If the cell concentration of any sample is higher than the maximum concentration of the counting method, dilute the cell suspension in cold Sample Buffer (Cat. No. 650000062) to within the recommended cell concentration range, and recount the cells. See Cell counting methods (page 62).

Preparing a single cell suspension for cartridge loading

Procedure

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

Number of captured cells on retrieved Cell Capture Beads (target)[a]	Estimated multiplet rate (%)
100	0.0
500	0.1
1,000	0.2
2,000	0.5
3,000	0.7

Number of captured cells on retrieved Cell Capture Beads (target)[a] (continued)	Estimated multiplet rate (%)
4,000	1.0
5,000	1.2
6,000	1.4
7,000	1.7
8,000	1.9
9,000	2.1
10,000	2.4
11,000	2.6
12,000	2.8
13,000	3.1
14,000	3.3
15,000	3.5
16,000	3.8
17,000	4.0
18,000	4.2
19,000	4.5
20,000	4.7

a. The number of cells sequenced might be less than the number of cells captured due to bead loss during handling, panel choice, and sample composition. The validated range of cells sequenced is 100–10,000 cells.

2. Determine the pooling ratio of samples to load onto the BD Rhapsody Cartridge. For example, if two samples were labeled using the BD Rhapsody™ Single-Cell Multiplexing Kit (Cat. No. 633781), and the samples will be pooled in equal proportion, the pooling ratio for each sample is 0.5. If only one sample is used, the pooling ratio is 1.

3. Calculate the volume, *V*, for each sample needed to prepare the pooled single cell suspension:

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

where:

V = volume of sample needed (µL)

N = desired number of captured cells in cartridge

P = pooling ratio

C = total cell concentration (cells/µL)

Example

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

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

P_A = sample *A* pooling ratio = 0.5

P_B = sample *B* pooling ratio = 0.5

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

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

$$\text{Volume of sample A needed} = 10{,}000 \text{ cells} \times 0.5 \times 1.36 / 200 \text{cells/µL} = 34 \text{ µL}$$

$$\text{Volume of sample B needed} = 10{,}000 \text{ cells} \times 0.5 \times 1.36 / 400 \text{cells/µL} = 17 \text{ µL}$$

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

$$Vn = 34\ \mu L + 17\ \mu L = 51\ \mu L$$

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

$$B = 650\ \mu L - 51\ \mu L = 599\ \mu L$$

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

6. According to the calculations in steps 2–5, prepare the cell suspension in cold Sample Buffer (Cat. No. 650000062) in a new 1.5 mL LoBind Tube.

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

7. If the samples were not filtered before counting cells, filter through a Falcon® Tube with Cell Strainer Cap (Corning Cat. No. 352235).

8. Proceed immediately to Processing cells with the BD Rhapsody™ Express Single-Cell Analysis system (page 73).

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6

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

Loading cells in the BD Rhapsody™ Cartridge

Best practices

- Always use low retention filtered pipette tips and LoBind Tubes.
- Perform single cell capture and cDNA synthesis in a pre-amplification workspace.
- Prepare cells as close to cell loading as possible. Keep the 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 49).
- 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. Load the cartridge on the tray with 700 µL of air using the BD Rhapsody P1200M pipette in **Prime/Treat** mode.
2. Change the mode of the BD Rhapsody P1200M pipette to **Cell Load**.
3. With a manual pipette, gently pipet the cell suspension up and down to mix. Immediately proceed to cell loading.

4. On the BD Rhapsody P1200M pipette, press the pipette button once to aspirate 40 µL of air, immerse the pipette tip in cell suspension, and then press the button again to aspirate 575 µL of cold cell suspension.
5. Insert the tip of the pipette perpendicular to the port, seal the pipette tip against the gasket, and then press the button a third time to dispense 615 µL of air and cells.

   Air bubbles that might appear at the inlet or outlet of the cartridge do not affect cartridge performance.
6. Leave the cartridge with loaded cells on the tray at room temperature (15°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.

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.

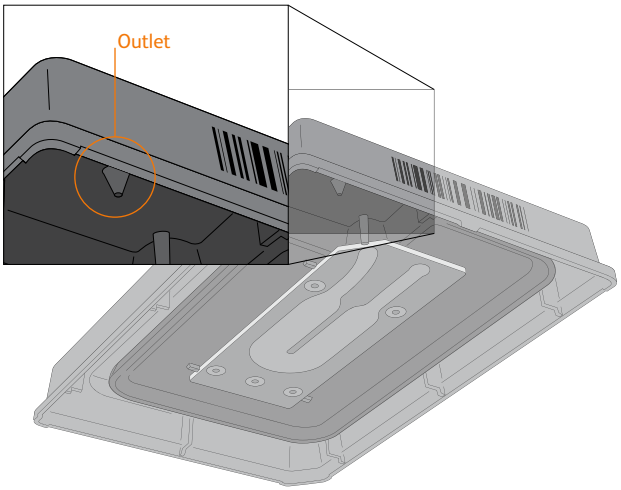
3. Remove the tube from the magnet, and then pipet 750 µL of cold Sample Buffer (Cat. No. 650000062) into the tube of beads.
4. Pipet the bead suspension up and down to mix.
5. Keep the beads on ice.

Loading Cell Capture Beads

Loading Cell Capture Beads on to the BD Rhapsody Cartridge

1. Change the mode of the BD Rhapsody P1200M pipette to **Prime/Treat**.
2. Load the cartridge with 700 µL of air using the BD Rhapsody P1200M pipette in **Prime/Treat** mode.
3. Change the mode of the BD Rhapsody P1200M pipette to **Bead Load**.
4. Use a P1000 standard pipette to gently pipet the 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 µL of beads.
5. Let the beads settle in the cartridge on the tray at room temperature (15°C to 25°C) for 3 minutes.
6. Place the cartridge on the plate shaker, and secure it on the plate adapter.

7. Shake the cartridge at room temperature (15°C to 25°C) for 15 seconds according to the settings for the plate shaker:
   - Eppendorf ThermoMixer® C: 1,000 rpm
   - Eppendorf MixMate®: 1,000 rpm
   - MicroPlate Genie®: 1,600 rpm. Set an external timer for 15 seconds.
8. Remove the cartridge from the plate shaker and, keeping the cartridge level, blot away the outlet drip from the bottom of the cartridge with a lint-free wiper:



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

    Note: In **Wash** mode, press the button once to aspirate 720 µL of air or reagent. After aspiration, insert the tip into the cartridge, and then press the button once to dispense 700 µL of air or liquid. After removing the pipette tip from the cartridge inlet, press the button once to dispense the remaining 20 µL of air or liquid before ejecting the pipette tip.

11. Load the cartridge with 700 µL of air using the BD Rhapsody P1200M pipette in **Wash** mode.
12. Load the cartridge with 700 µL of cold Sample Buffer (Cat. No. 650000062) using the BD Rhapsody P1200M pipette in **Wash** mode.
13. Repeat steps 11–12 once for a total of two washes.

Lysing cells and retrieving Cell Capture Beads

Lysing the cells

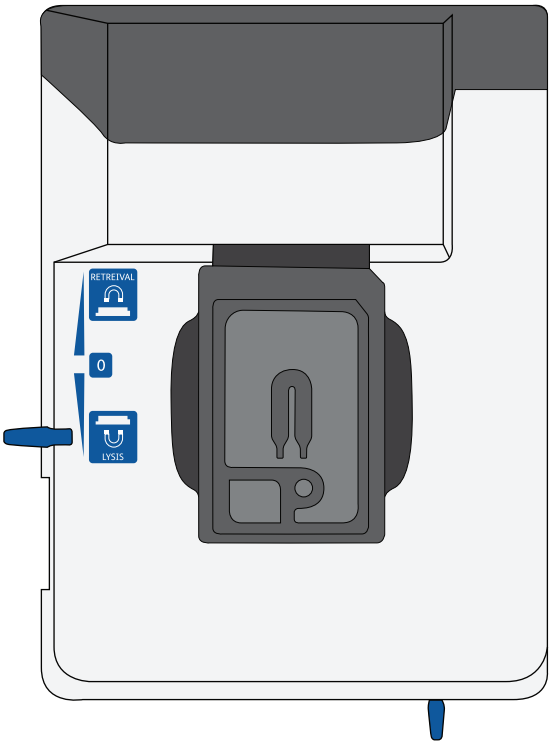
Avoid bubbles.

1. Add 75.0 µL 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 ≤24 hours, and then discard.

2. Briefly vortex the lysis mix, and place it on ice.

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

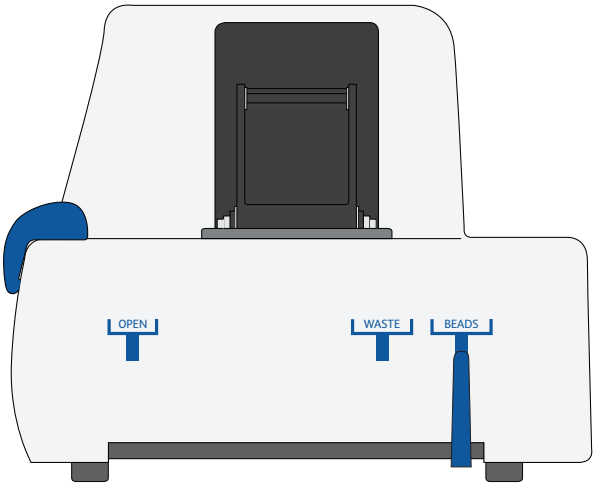


4. Change the mode on the BD Rhapsody P1200M pipette to **Lysis**.
5. Load the cartridge with 550 µL of Lysis Buffer with DTT using the BD Rhapsody P1200M pipette in **Lysis** mode.
6. Leave the cartridge on the tray at room temperature (15°C to 25°C) for 2 minutes.

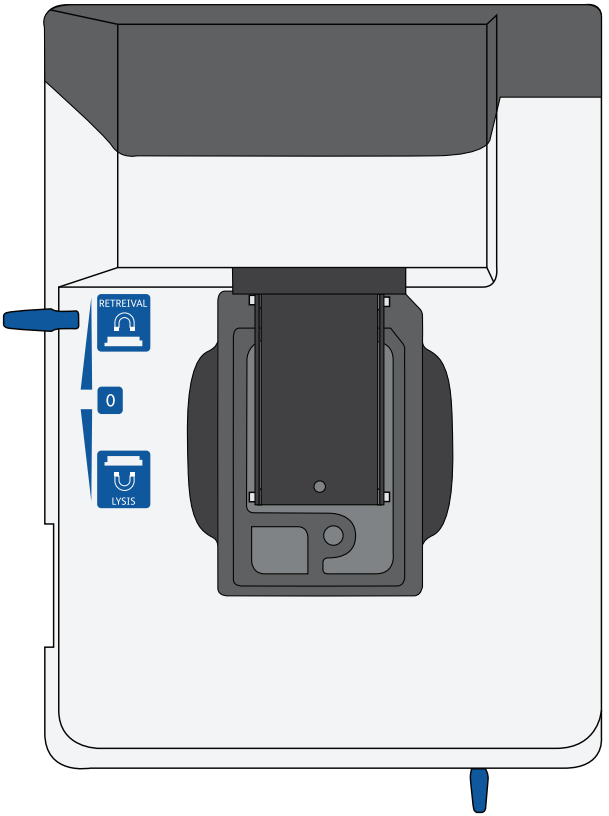
   Maintain recommended lysis time for best performance.

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 µL of Lysis Buffer with DTT.
7. Press down firmly on the BD Rhapsody P5000M pipette to seal the pipette tip against the gasket of the cartridge to avoid leaks.

8. Move the left slider to the middle (**0**) position, and *immediately* load the cartridge with 4,950 µL of Lysis Buffer with DTT using the BD Rhapsody P5000M pipette. The Retrieval (top) magnet is in its full up position and is away from the cartridge.

   The Cell Capture Beads are collected in the 5 mL LoBind Tube.

9. Remove the pipette tip from the inlet gasket of the cartridge before pressing the dial button once to purge the tip. Discard the pipette tip.

10. Move the front slider to **OPEN**, and then remove and cap the 5 mL LoBind Tube.

11. Uncap the tube, and place it on the large magnetic separation stand fitted with the 15 mL tube adapter for 1 minute.

12. Proceed immediately to Performing reverse transcription on the Cell Capture Beads (page 84) to process the beads and begin reverse transcription.

13. Appropriately dispose of the BD Rhapsody Cartridges according to biosafety level (BSL):

	**Biological hazard**

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

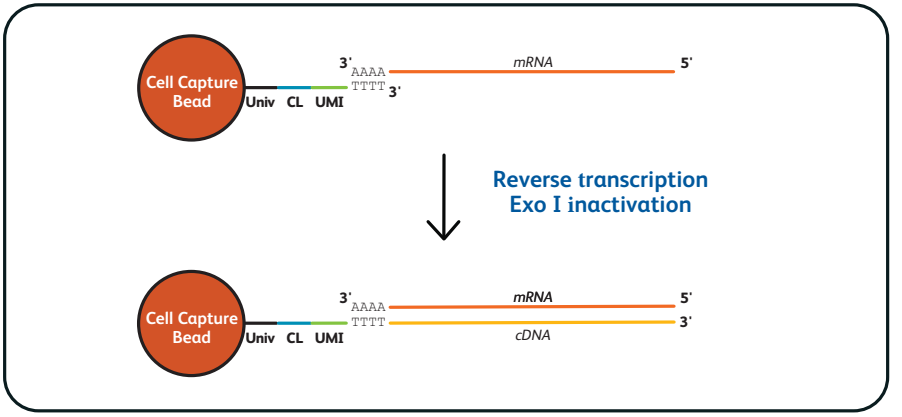
   Dispose of waste using proper precautions and in accordance with local regulations. For more information, see Waste (page 124).

14. Appropriately dispose of the waste in the Waste Collection Container.
15. Appropriately dispose of the Lysis Buffer with DTT.
16. Wipe the BD Rhapsody Express instrument with 10% (v/v) bleach or 70% (v/v) ethyl alcohol. See the *BD Rhapsody™ Express Single-Cell Analysis System Installation and Maintenance Guide for the BD Rhapsody™ Express Instrument* (Doc ID 76955).

Performing reverse transcription on the Cell Capture Beads

Introduction

Prepare the reverse transcription mix, wash the Cell Capture Beads (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 ≤30 minutes after washing retrieved beads with Bead Wash Buffer.

Before you begin

- Obtain the 5 mL LoBind Tube of retrieved beads. See Lysing cells and retrieving Cell Capture Beads (page 79).
- 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 Lysing cells and retrieving Cell Capture Beads (page 79)] and while leaving the 5 mL LoBind Tube on the large magnet, use a pipette to carefully remove all but ~1 mL of supernatant without disturbing the beads.
2. Remove the tube from the large magnet, resuspend the ~1 mL beads by gently pipetting the suspension up and down, and then transfer the bead suspension to a new 1.5 mL LoBind Tube.
3. If the beads remain into the 5 mL LoBind Tube, pipet an additional 0.5 mL of Lysis Buffer with DTT into the 5 mL tube, rinse the 5 mL tube, and transfer the suspension to the 1.5 mL LoBind Tube of beads.
4. Place the tube on the 1.5 mL tube magnet for ≤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 ≤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 ≤30 minutes after washing retrieved beads with Bead Wash Buffer.

Performing reverse transcription

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

Limit the preparation of mixes to ≤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, 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
Total	**200.0**	**240.0**

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

4. Place the tube of washed beads [see Washing the Cell Capture Beads (page 85)] on the 1.5 mL tube magnet for ≤2 minutes, and then carefully remove and appropriately discard the supernatant without disturbing the beads and while leaving the tube on the magnet.
5. Use a low retention tip to pipet 200 µL of the cDNA mix to resuspend the beads. Gently mix the suspension by pipette only. Do not vortex.

   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. Ensure that the SmartBlock Thermoblock 1.5 mL or equivalent is installed on the thermomixer.
8. Incubate the suspension on the thermomixer at 1,200 rpm and 37°C for 20 minutes.

   Shaking is critical for this incubation.
9. 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 the preparation of mixes to ≤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 by adding the components in the following order:

   Exonuclease I mix

Component	1 library (µL)	1 library + 20% overage (µL)
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
Total	**200.0**	**240.0**

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

5. Remove the tube from the magnet, and with a low retention tip, pipet 200 µL of cold Bead Resuspension Buffer (Cat. No. 650000066) to gently resuspend the beads. Do not vortex.

   Stopping point: The treated beads can be stored at 2°C to 8°C for ≤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.

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7

Troubleshooting

Introduction

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

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

Troubleshooting is organized as follows:

Troubleshooting by microscopy: introduction

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

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

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

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

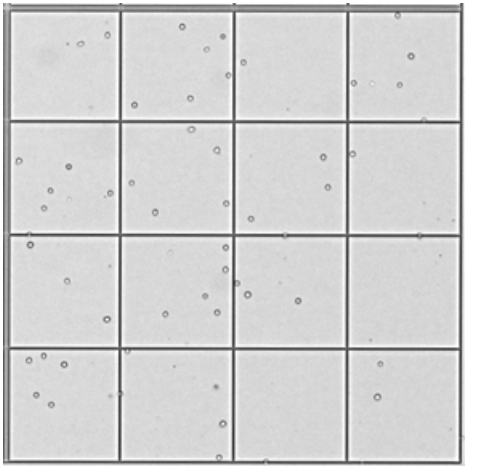
After having identified specific issues with cartridge loading by microscopy, proceed to the tables of observations for additional recommended solutions. See Cartridge preparation troubleshooting (page 111).

Troubleshooting by microscopy: cell preparation of single and multiplexed samples

Example image of cells in hemocytometer

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

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



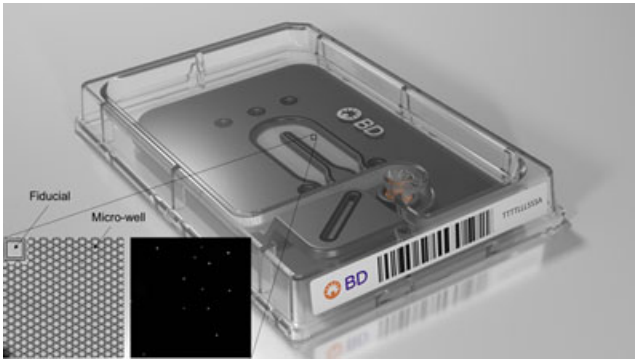
Troubleshooting by microscopy: cartridge inspection after Cell Load

Introduction

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

Estimate cell viability and the total number of cells captured

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



3. Image the BD Rhapsody Cartridge under a microscope taking brightfield and fluorescence images.

4. In the field of view under the microscope, count and record the number of:
   - Calcein AM positive cells
   - Propidium Iodide positive cells
   - Wells in the field of view

   Note: If the count of Calcein AM positive cells is <100, count cells in another field of view until the Calcein AM count is ≥100.

5. Estimate cell viability and the total number of cells captured in the cartridge:

$$\text{Viability} = \text{No. Calcein AM positive cells} / (\text{No. Calcein AM positive cells} + \text{no. of PI positive cells})$$

$$\text{Total no. cells captured} = \text{cells/well} \times 221{,}891$$

Example

In a field of view of the cartridge wells, which is arbitrarily called Region 1 [see **Region 1: 26 cells (page 99)**], 26 cells are counted in 225 wells (15 rows × 15 columns of microwells). To count a total of ≥100 cells, an additional four regions are counted [see **Region 2: 14 cells (page 100)** through **Region 5: 26 cells (page 101)**]:

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

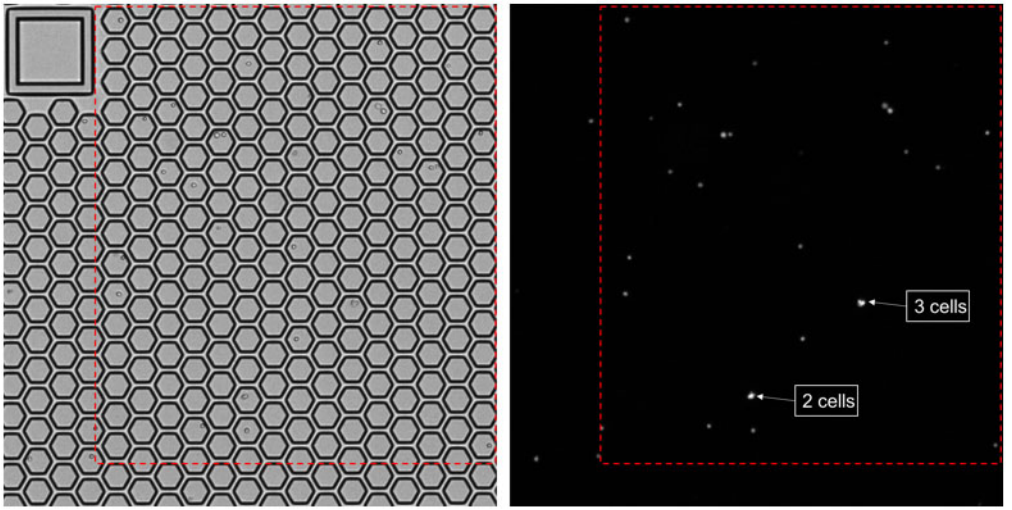
Therefore, accounting for five regions:

$$\text{Cells/well} = 105/1,125 = 0.0933$$

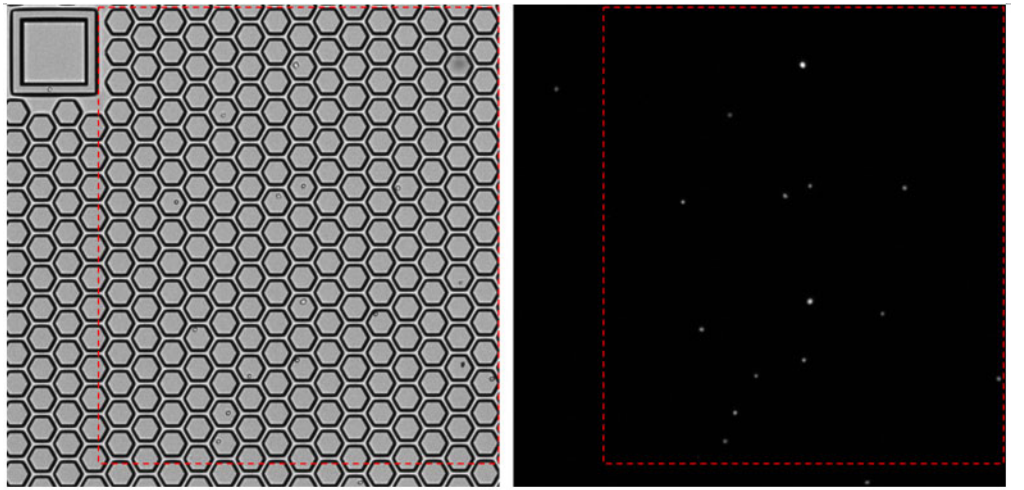
$$\text{Total no. cells captured in the cartridge} = 0.0933 \times 221{,}891$$

$$\text{Total no. cells captured in the cartridge} = 20,702$$

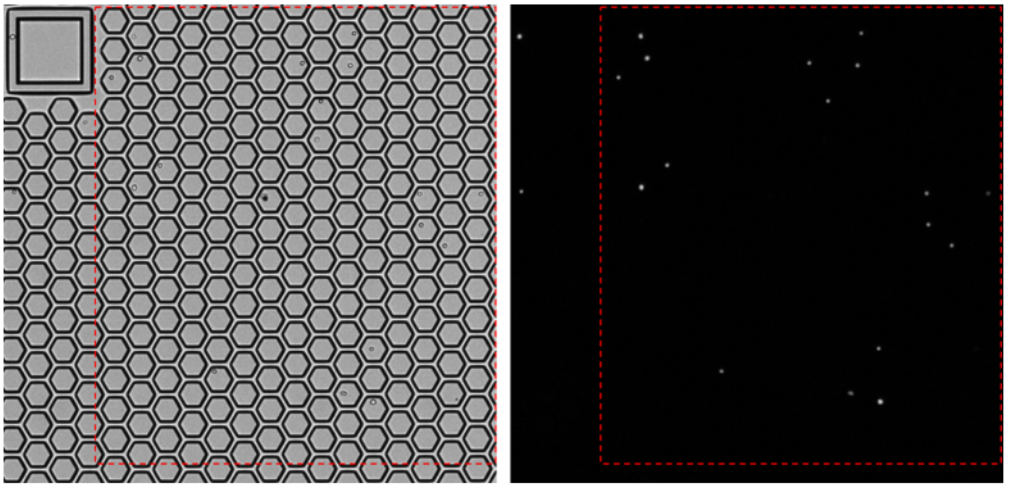
Region 1: 26 cells



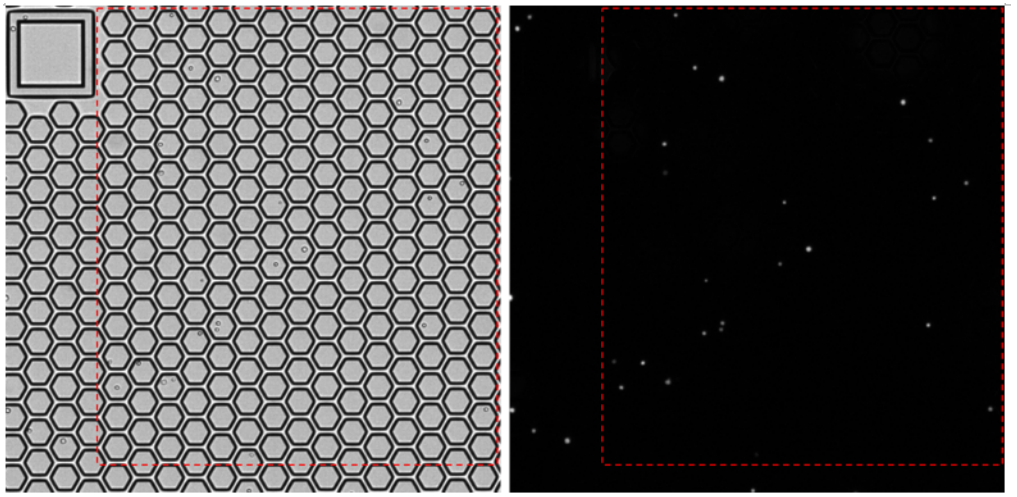
Region 2: 14 cells



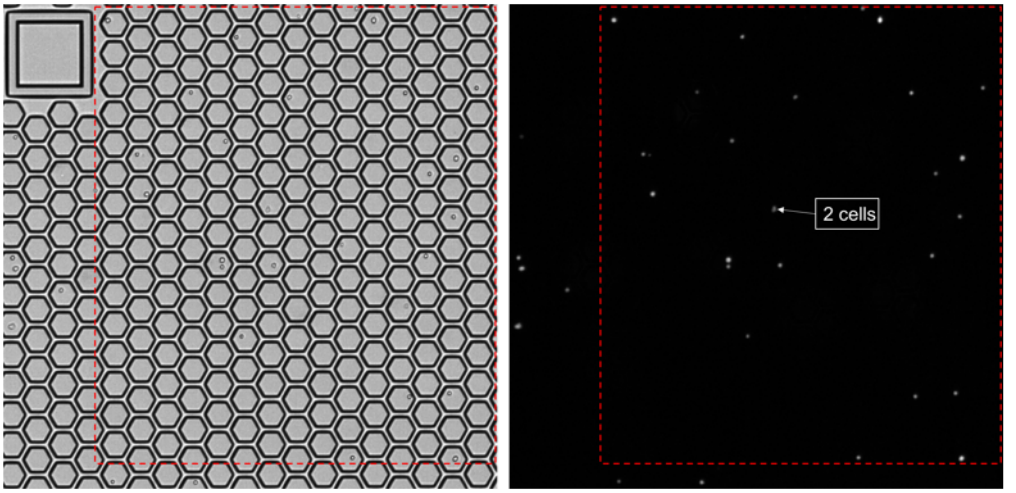
Region 3: 17 cells



Region 4: 22 cells



Region 5: 26 cells



Troubleshooting by microscopy: cartridge inspection after Bead Load

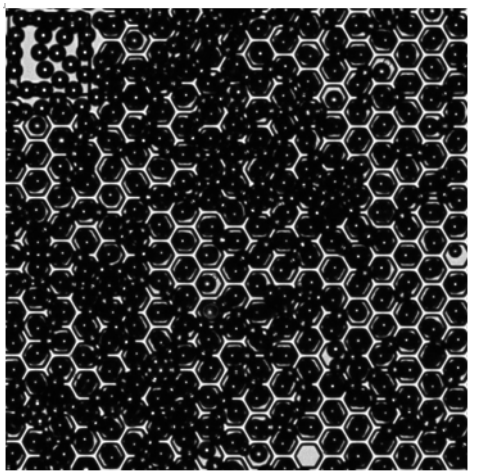
Introduction

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

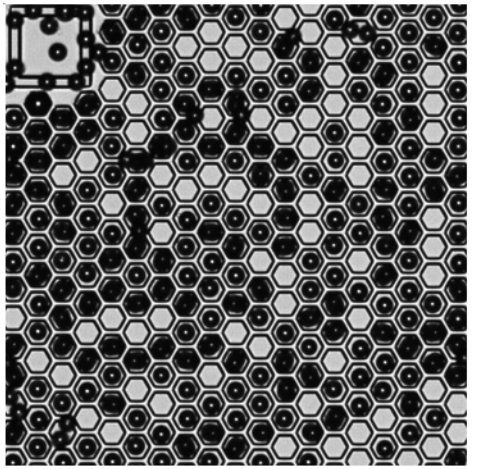
Example images after Bead Load

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

Acceptable loading of Cell Capture Beads after bead loading



Insufficient loading of Cell Capture Beads after bead loading



Troubleshooting by microscopy: cartridge inspection after Bead Wash

Introduction

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

Estimate the bead loading efficiency and number of captured cells on Cell Capture Beads

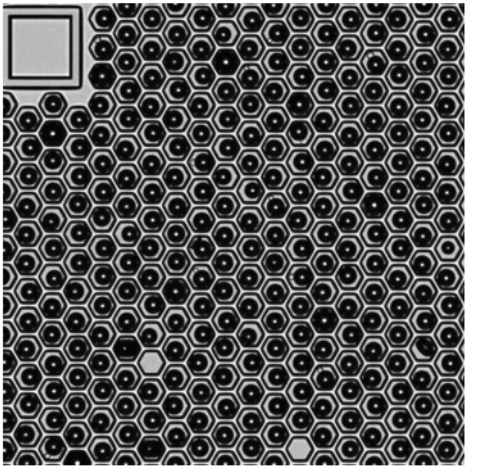
1. After the Bead Wash step is complete, image the BD Rhapsody Cartridge under a microscope. Brightfield microscopy is required, and fluorescence microscopy is recommended.
2. Note the location of the image within the microwell array by recording the location of the fiducial (post) in order to image the cartridge at the same location.
3. Calculate the bead loading efficiency:
   a. Count the number of beads in ≥200 wells.
   b. Calculate:
   Bead loading efficiency = No. wells containing one bead/no. wells
4. Estimate the total number of cells captured in the cartridge. See Estimate cell viability and the total number of cells captured (page 97). You will use the total number of cells captured in the cartridge in step 6.
5. Use the concentration of cell suspension at Cell Load to find the estimated multiplet rate. See the multiplet rate table in Preparing a single cell suspension for cartridge loading (page 68). You will use the estimated multiplet rate in step 6.
6. Estimate the number of captured cells on Cell Capture Beads:

$$\text{No. captured cells on beads} = (\text{Bead loading eff} \times \text{no. cells captured} \times (100 - \text{est multiplet rate})/100)$$

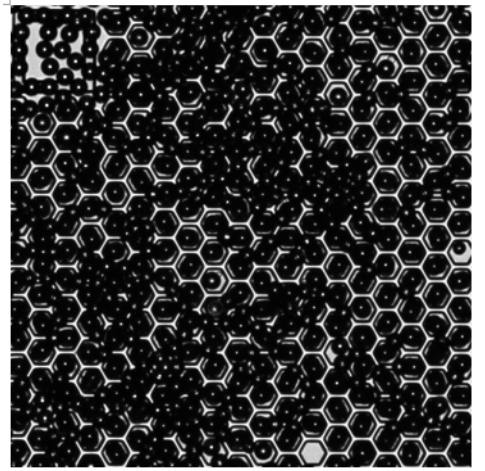
Example images after Bead Wash

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

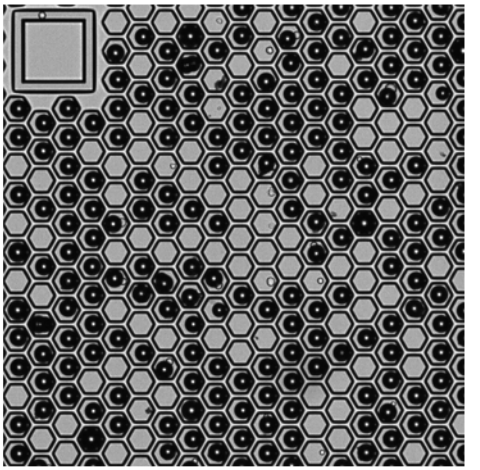
Acceptable bead number after the second bead wash



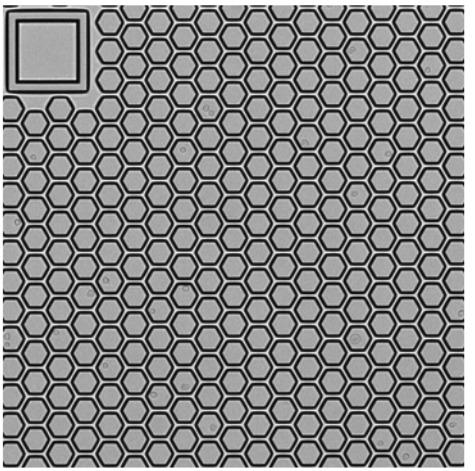
Insufficient removal of excess Cell Capture Beads



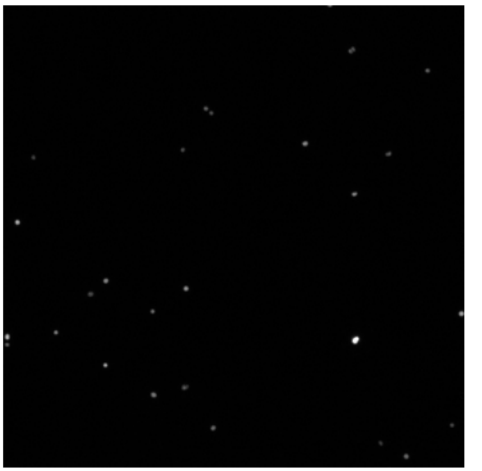
Significant loss of Cell Capture Beads resulting in empty microwells after the second bead wash



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

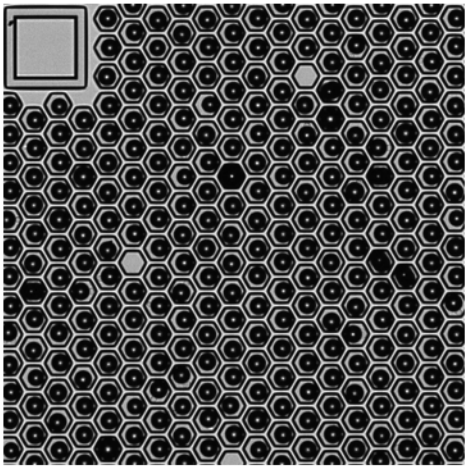


Brightfield



Fluorescent

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



Brightfield

Fluorescent

Troubleshooting by microscopy: cartridge inspection after Bead Retrieval

Introduction

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

Estimate the number of remaining beads after Bead Retrieval

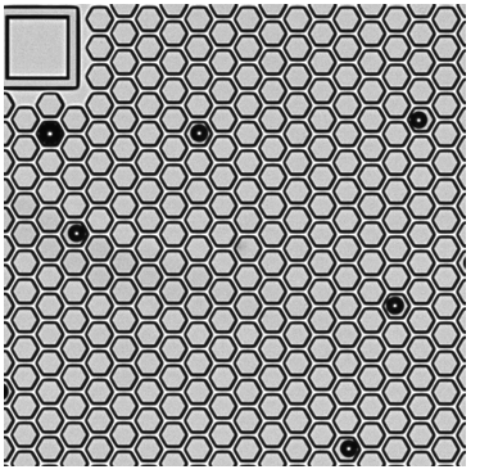
1. After bead retrieval is complete, image the BD Rhapsody Cartridge under a brightfield microscope. Quantities of beads observed in brightfield images might indicate insufficient bead retrieval.
2. Calculate the remaining beads in the cartridge after bead retrieval:
   a. Count the number of beads in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

$$\text{Percent remaining beads} = \text{No. wells containing one bead} / (\text{No. wells})$$

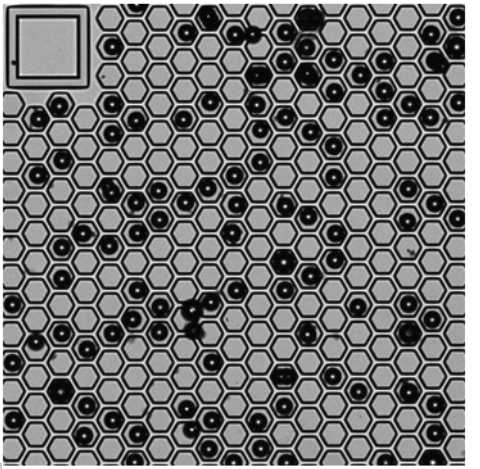
Example images after Bead Retrieval

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

Acceptable retrieval of Cell Capture Beads



Poor retrieval of Cell Capture Beads



Troubleshooting by microscopy: BD Rhapsody Cartridge handling

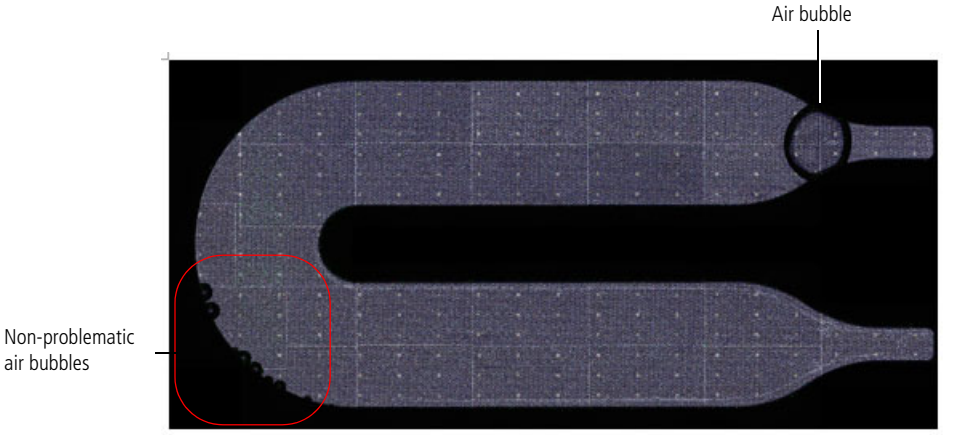
Introduction

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

Example image of the BD Rhapsody Cartridge

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

Air bubble in cartridge after Bead Wash. In this brightfield example, the yield of final retrieved cell capture beads would decrease by ~3%. Check the cartridge after each workflow step:



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

Cartridge preparation troubleshooting

Introduction

First, perform troubleshooting by microscopy to identify issues with the cartridge workflow before using the cartridge preparation troubleshooting tables in this section. See Troubleshooting by microscopy: introduction (page 95). Next, match identified issues with the listed observations in the tables and follow the recommended solutions.

After Cell Load

Observation	Possible causes	Recommended solutions
Cell viability is significantly lower than the viability observed during cell preparation	Suboptimal sample quality and/or sample handling	• Remove dead cells by standard procedures. • Keep cells on ice. • Reduce the time from cell preparation to cell loading in the BD Rhapsody Cartridge.
Estimated cells captured is significantly higher than the estimated cells loaded	• Incorrect cell concentration • Incorrect dilution	• Recount the cells or use a different counting method. **Recounting and recalculating would apply to a new cartridge. Proceed at own risk with current cartridge.** • Recalculate the cell dilution.
	Improper cell counting	Follow best practices for cell counting. See Best practices for cell handling and cell counting (page 60).

Observation (continued)	Possible causes	Recommended solutions
Estimated cells captured is significantly lower than the estimated cells loaded	• Incorrect cell concentration • Incorrect dilution	• Recount the cells or use a different counting method. **Recounting and recalculating would apply to a new cartridge. Proceed at own risk with current cartridge.** • Recalculate the cell dilution.
	Improper cell counting	Follow best practices for cell counting. See Best practices for cell handling and cell counting (page 60).
	Cell diameter >20 µm	The percentage of cells recovered in sequencing declines for cells >20 µm in diameter. Load additional cells to offset the losses observed.

After Bead Load

Observation	Possible causes	Recommended solutions
Too few beads loaded	Incorrect preparation of Cell Capture Beads	• Confirm underloading of beads with example image. See **Acceptable loading of Cell Capture Beads after bead loading (page 103)**. • Pellet Cell Capture Beads and resuspend them in Sample Buffer (Cat. No. 650000062). • Thoroughly resuspend beads before loading into cartridge.
	Wrong pipette mode	Use the **Bead Load** mode on the BD Rhapsody™ P1200M pipette.

After bead wash

Observation	Possible causes	Recommended solutions
Observed cell multiplets are significantly higher than the expected multiplet rate	Cell samples that tend to clump	Filter the cell suspension through an appropriately sized cell strainer multiple times to remove clumps and doublets.
Insufficient removal of excess Cell Capture Beads	Bead wash steps missed	• Confirm excess beads with images. See **Acceptable bead number after the second bead wash (page 105)**. • Repeat the two bead wash steps after shaking the cartridge on the plate shaker.
	Wrong pipette mode	Use the **Wash** mode on the BD Rhapsody P1200M pipette for the two bead wash steps.

Observation continued	Possible causes	Recommended solutions
Estimated bead load efficiency is <80%	Cartridge shaking step skipped	• Confirm loss of cells with images. See **Acceptable bead number after the second bead wash (page 105)** and **Significant loss of Cell Capture Beads resulting in empty microwells after the second bead wash (page 106)**. • Repeat the experiment with a new cartridge. Ensure that the cartridge is shaken on the plate shaker before the two bead wash steps.
	Wrong plate shaker settings	Repeat the experiment with a new cartridge. Ensure that the plate shaker settings are correct.
	Wrong pipette mode	Repeat the experiment with a new cartridge. Use the **Wash** mode on the BD Rhapsody P1200M pipette for the two bead wash steps.

Observation continued	Possible causes	Recommended solutions
Significant loss of cells at Bead Wash	Cell viability low	• Confirm loss of cells with images. See After Bead Retrieval and **Significant cell loss after the second bead wash: Images after the second bead wash (page 107)**. • Try to ensure that cell viability is ≥50%.
	Incorrect removal of buffer from the cartridge through pipette aspiration	Only use the pipette in the correct mode to dispense air into the cartridge to remove buffer.
	Wrong pipette mode	Use the correct pipette mode at every step.

After Bead Retrieval

Observation	Possible causes	Recommended solutions
Remaining beads >10% of wells with beads	Retrieval (top) magnet not in down position during retrieval step	• Confirm poor retrieval of beads with images. See **Poor retrieval of Cell Capture Beads (page 109)**. • Ensure that the retrieval magnet is in the down position for 30 seconds before retrieving the Cell Capture Beads.
	Wrong pipette mode	Use the **Retrieval** mode on the BD Rhapsody™ P5000M pipette.

Cartridge handling

Observation	Possible causes	Recommended solutions
Dropped the cartridge or hit it against an object	Various	• If the cartridge was dropped, BD Biosciences recommends using a new cartridge. • If the cartridge was struck, proceed at your own risk.
Air bubble in cartridge	Air bubble present in pipette tip while dispensing buffer	Ensure that the pipette tip contains only buffer and no air bubble is trapped at the end of aspiration of buffer. **Cells will not be lysed and beads will not be retrieved under the bubble.**
	Re-used pipette tip	Use a new pipette tip at every pipetting step.

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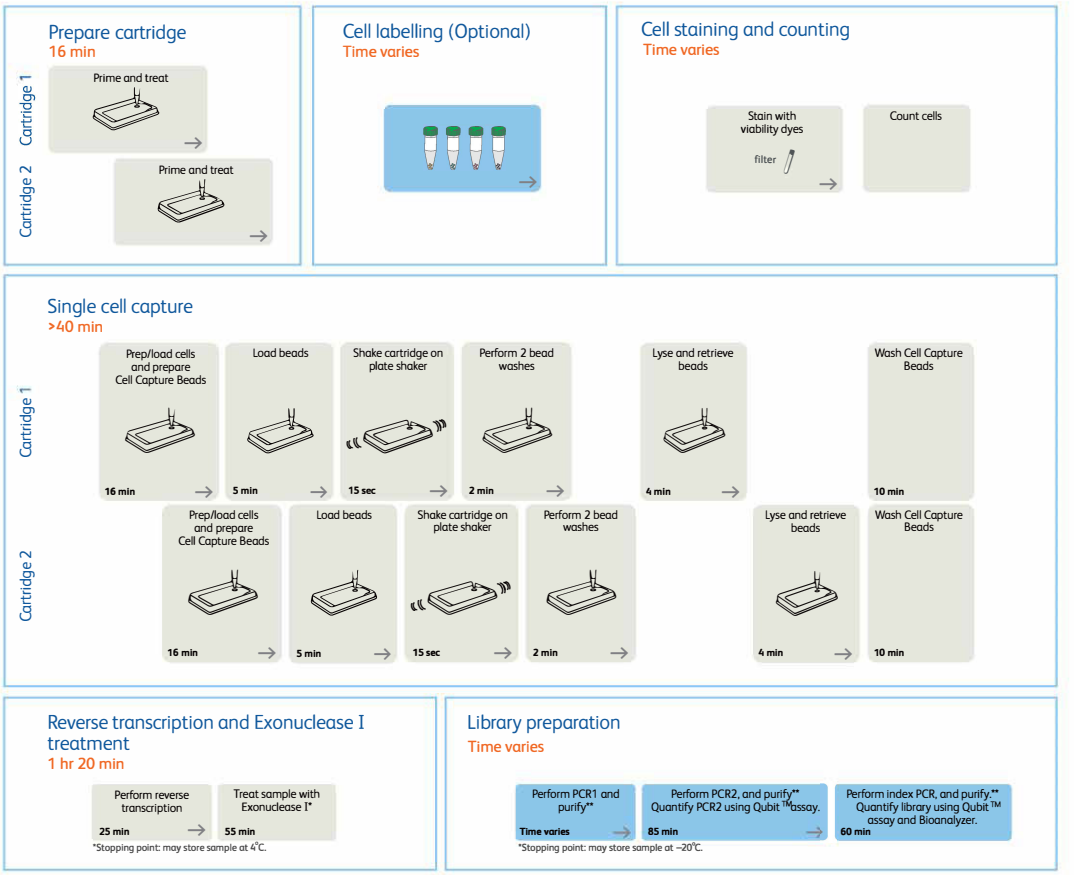
A

Workflow with two BD Rhapsody™ Cartridges

Workflow with two BD Rhapsody Cartridges

Staggered boxes indicate staggering the start of like steps.



Best practices with a two-cartridge workflow

Reagent preparation

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

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

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B

Safety

General safety and limitations

For instrument safety, see the *BD Rhapsody™ Express Instrument Safety and Limitations Guide* (Doc ID: 76918).

Genomics technical publications are available for download from the BD Genomics Resource Library at bd.com/genomics-resources.

Chemical safety

Requirements

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

Waste

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

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

Physical safety

See the *BD Rhapsody™ Express Instrument Safety and Limitations Guide* (Doc ID: 76918).

Genomics technical publications are available for download from the BD Genomics Resource Library at bd.com/genomics-resources.

Instrument waste disposal

Disposal of the instrument

Contact BD Biosciences technical support at researchapplications@bd.com before disposing of the BD Rhapsody™ Express instrument. For more information, see Instrument technical support (page 13).

Glossary

B

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

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

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

L

load
To add a reagent to the BD Rhapsody Cartridge.