BD Rhapsody™ System

Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Express Single-Cell Analysis System

Protocol

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Regulatory information

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History

Revision	Date	Change made
Doc ID: 210967 Rev. 1.0	2018-07	Initial release.
23-22952-00 (Doc ID: 210967 Rev. 2.0)	2020-07	Assigned new 23 document part number, updated catalog number for reverse transcriptase, recommended thermomixer instead of heat block for Exonuclease I inactivation.
23-22952(01) (Doc ID: 210967 Rev. 3.0)	2022-01	Added BD Rhapsody™ Enhanced Cell Capture Beads and part numbers.
23-22952(02)	2022-11	Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers. Added note at the end of the Washing BD Rhapsody™ Enhanced Cell Capture Beads workflow: **Note:** If performing the TCR and/or BCR assay, stop here and proceed with respective protocol to continue cDNA synthesis.

Contents

Introduction

This protocol describes cell loading in the BD Rhapsody™ Cartridge and single-cell capture with the BD Rhapsody™ Express Single-Cell Analysis System.

For complete instrument procedures, safety information, and workflow, see the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide*.

Required and recommended materials

For a complete list of materials, see the instrument user guide.

Required reagents

Material	Supplier	Catalog no.
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
BD Rhapsody™ Cartridge Kit	BD Biosciences	633733
BD Rhapsody™ cDNA Kit	BD Biosciences	633773
Absolute ethyl alcohol, molecule biology grade	Major supplier	–
Nuclease-free water	Major supplier	–

Recommended consumables

Material	Supplier	Catalog no.
Gilson™ PIPETMAN™ Tipack™ Filter Tips, 100-1200 µL for BD Rhapsody™ P1200M pipette	Thermo Fisher Scientific	F171803G
Gilson™ PIPETMAN™ Tipack™ Filter Tips, 500-5000 µL for BD Rhapsody™ P5000M pipette	Thermo Fisher Scientific	F161370G
Falcon® Tube with Cell Strainer Cap	Corning	352235
DNA LoBind® Tubes, 1.5-mL	Eppendorf	30108051
DNA LoBind® Tubes, 5.0-mL[a]	Eppendorf	30108310
Low-retention, filtered pipette tips (10 µL, 200 µL, 1000 µL)	Major supplier	–
Pre-moistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol.	Major supplier	–
Lint-free wipes	Major supplier	–
a. These are the Bead Retrieval Tubes to be used with the BD Rhapsody™ Express instrument.		

Equipment

Material	Supplier	Catalog no.
BD Rhapsody™ Express Instrument[a]	BD Biosciences	633702
BD Rhapsody™ P1200M pipette[a]	BD Biosciences	633704
BD Rhapsody™ P5000M pipette[a]	BD Biosciences	633705
Cell Counter	Major supplier	–
Large magnetic separation stand	V&P Scientific, Inc.	VP 772FB-1
Clear acrylic cylinder adapter for 15-mL tube adapter[b]	V&P Scientific, Inc.	VP 772FB-1A
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Centrifuge and rotor with adapters for 5mL Falcon® tubes and 15-mL tubes	Major supplier	–
Eppendorf ThermoMixer® C	Eppendorf	5382000023
SmartBlock™ Thermoblock 1.5-mL	Eppendorf	5360000038
Plate Shaker for cartridge workflow: SmartBlock™ plates (for ThermoMixer C) Or, Eppendorf MixMate® Or, MicroPlate Genie™	 Eppendorf Eppendorf Scientific Industries, Inc.	 5363000039 22674200 SI-0400
Water bath OR incubator at 37 °C	Major supplier	–
Pipettes (P10, P20, P200, P1000)	Major supplier	–
Vortexer	Major supplier	–
Digital timer	Major supplier	–
6-Tube Magnetic Separation Rack for 1.5-mL tubes Or, 12-Tube Magnetic Separation Rack Or, Invitrogen™ DynaMag™-2 Magnet	New England Biolabs New England Biolabs Thermo Fisher Scientific	S1506S S1509S 12321D

a. Part of the BD Rhapsody™ Single-Cell Analysis system. Items can be ordered separately.
b. Holds 5-mL LoBind tube in magnet.

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, on ice unless instructed otherwise.
- Change pipette tips before every pipetting step.
- 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.

Before you begin

- Thaw reagents (not enzymes) in the BD Rhapsody™ cDNA Kit at room temperature (15–25 °C), and then place on ice. Keep enzymes at –25 °C to –15 °C.
- Place on ice the following components of the BD Rhapsody™ Enhanced Cartridge Reagent Kit:
  - Sample Buffer
  - 1 M DTT
  - Lysis Buffer
  - BD Rhapsody™ Enhanced Cell Capture Beads
- Ensure that the SmartBlock™ Thermoblock 1.5 mL or equivalent is installed on the thermomixer and is set to 37 °C for 20 minutes.
- Set an additional thermomixer to 80 °C if available.
- Prepare a single-cell suspension. See *Preparing Single-Cell Suspensions Protocol*.
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single-Cell Suspensions Protocol*.
- Visually inspect the Lysis Buffer for any precipitation. If precipitation is present, incubate the Lysis Buffer at room temperature for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, place the Lysis Buffer on ice.
- Open the tube while holding the DTT tube vertically. The solution is overlain with an inert/non-oxygen-containing gas and a non-vertical tube will allow the inert gas to pour off. If not loading four cell cartridges at the same time, after opening the DTT tube once, seal and store the tube at –20 °C.

Priming and treating the BD Rhapsody™ Cartridge

Prime and treat the BD Rhapsody™ Cartridge. For detailed instructions, see the instrument user guide.

Express instrument slider	Position
Front	Waste
Side	0

Step no.	Material to load	Volume (µL)	P1200M pipette mode	Incubation at room temperature
1	100% ethyl alcohol	700	Prime/Treat	—
2	Air	700	Prime/Treat	—
3	Room temp. Cartridge Wash Buffer 1	700	Prime/Treat	1 min
4	Air	700	Prime/Treat	—
5	Room temp. Cartridge Wash Buffer 1	700	Prime/Treat	10 min
6	Air	700	Prime/Treat	—
7	Room temp. Cartridge Wash Buffer 2	700	Prime/Treat	≤4 hr

Counting and preparing a single-cell suspension for cartridge loading

For detailed instructions, see the instrument user guide.

1 Treat the cells with viability stain(s), and count. Order of accurate counting:
   - Manual counting with fluorescence
   - Automated counting with fluorescence
   - Automated counting with Trypan Blue Stain and brightfield
   - Manual counting with Trypan Blue Stain and brightfield

2 Determine the desired number of cells to capture in the BD Rhapsody™ Cartridge . See the instrument user guide for a table containing estimated multiplet rates based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads.

3 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, 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.

4 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

Volume of sample A needed = 10,000 cells × 0.5 × 1.36/200cells/µL= 34 µL

Volume of sample B needed = 10,000 cells × 0.5 × 1.36/400cells/µL= 17 µL

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

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

6 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 5**:

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

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

7 According to the calculations in steps 3–6, prepare the cell suspension in cold Sample Buffer in a new 1.5-mL LoBind tube.

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

8 If the samples were not filtered before counting the cells, filter through a Falcon® tube with a Cell Strainer Cap.

Loading cells in the cartridge

1 Load the cartridge with materials listed using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Prime/Treat
• Set P1200M pipette to **Cell Load** mode. • Pipet-mix the cell suspension with a manual P1000 pipette.		
Cell suspension	575	**Cell Load**[a]
a. Press button once to aspirate 40 µL air, and then immerse tip in cell suspension. Press button again to aspirate 575 µL of cold cell suspension. Dispense 615 µL of air and cell suspension.		

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

2 If necessary, wipe condensation from the top cartridge surface for optimal scanning.

3 Incubate at room temperature (15–25 °C) for 15 minutes.

During the 15-minute incubation, prepare the BD Rhapsody™ Enhanced Cell Capture Beads. See Preparing BD Rhapsody™ Enhanced Cell Capture Beads in the following section.

Preparing BD Rhapsody™ Enhanced Cell Capture Beads

Keep BD Rhapsody™ Enhanced Cell Capture Beads on ice before use.

For maximum recovery, do not vortex samples containing BD Rhapsody™ Enhanced Cell Capture Beads. Gently mix suspensions with BD Rhapsody™ Enhanced Cell Capture Beads by pipette only. Use low-retention pipette tips and LoBind tubes. Keep the beads cold, and pipet-mix only.

1 Place the BD Rhapsody™ Enhanced Cell Capture Beads tube on the magnet for 1 minute, and remove the storage buffer.

2 Remove the tube from the magnet, and pipet 750 µL of cold Sample Buffer into the tube.

3 Pipet-mix, and place on ice.

Loading and washing BD Rhapsody™ Enhanced Cell Capture Beads

1 Set the P1200M pipette to **Prime/Treat** mode.

2 Load the cartridge with the materials listed using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	**Prime/Treat**
• Set P1200M pipette to **Bead Load** mode. • Use a manual P1000 to gently pipet-mix the beads in cold Sample Buffer. Immediately load.		
BD Rhapsody™ Enhanced Cell Capture Beads	630	**Bead Load**

3 Incubate the cartridge at room temperature (15–25 °C) for 3 minutes.

4 Place the cartridge on the plate shaker plate adapter.

5 Shake the cartridge at room temperature (15–25 °C) for 15 seconds.

Following is a list of suggested shakers/mixers with their corresponding settings:

- Eppendorf ThermoMixer® C: 1,000 rpm
- Eppendorf MixMate®: 1,000 rpm
- MicroPlate Genie®: 1,600 rpm. Set external timer to 15 seconds.

6 Blot the outlet drip with a lint-free wipe.

7 Return the cartridge to the Express instrument, and wait 30 seconds.

8 Set the P1200M pipette to **Wash** mode.

9 Load the cartridge with the following materials using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode[a]
Air	700	Wash
Cold Sample Buffer	700	Wash
Air	700	Wash
Cold Sample Buffer	700	Wash

a. Press button once to aspirate 720 µL air or reagent. Insert the tip into the cartridge, and press button once to dispense 700 µL air or liquid. Remove pipette tip, and press button once to dispense remaining 20 µL of air or liquid.

Lysing cells

Avoid bubbles.

1 Add 75.0 µL of 1 M DTT to one 15-mL Lysis Buffer bottle. Check Add DTT box.

   Use the Lysis Buffer with DTT ≤24 hours, and then discard.

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

3 Move the left slider to LYSIS on the Express instrument.

4 Set the P1200M pipette to **Lysis** mode.

5 Load the cartridge with the following material using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Lysis Buffer with DTT	550	Lysis

6 Incubate at room temperature (15–25 °C) for 2 minutes.

   Maintain the recommended lysis time for best performance.

Retrieving BD Rhapsody™ Enhanced Cell Capture Beads

1. Place the 5-mL LoBind tube in the Express instrument drawer.
2. Ensure that the P5000M pipette is set to **Retrieval** mode.
3. Move the front slider to **BEADS** on the Express instrument.
4. Move the left slider to **RETRIEVAL**.
5. Leave the Retrieval magnet in the down position for 30 seconds.
6. Aspirate 5,000 µL of Lysis Buffer with DTT using the P5000M pipette.
7. Press down on the P5000M pipette to seal against the gasket.
8. Move the left slider to the middle position (0), and *immediately* load 4,950 µL of Lysis Buffer with DTT.
9. Remove the pipette from the gasket, and purge the tip.
10. Move the front slider to **OPEN**, and place the 5-mL LoBind tube on the large magnet with the 15-mL tube adapter for 1 minute.
11. Immediately proceed to Washing BD Rhapsody™ Enhanced Cell Capture Beads in the following section.
12. Appropriately dispose of the cartridge, Waste Collection Container, and Lysis Buffer with DTT.

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.

13. Clean the Express instrument with 10% bleach or 70% ethyl alcohol.

Washing BD Rhapsody™ Enhanced Cell Capture Beads

1. After the 1-minute incubation, leaving the 5-mL tube containing retrieved BD Rhapsody™ Enhanced Cell Capture Beads on the large magnet, remove all but ~1 mL of supernatant without disturbing the beads.
2. Remove the tube from the magnet. Gently pipet-mix the beads and transfer them to a new 1.5-mL LoBind tube.
3. If there are still beads left in the 5-mL tube, add 0.5 mL of Lysis Buffer with DTT, rinse the 5-mL tube, and transfer to the 1.5-mL LoBind tube from the previous step.
4. Place the tube on the 1.5-mL magnet for ≤2 minutes. Remove the supernatant.

   Avoid leaving Lysis Buffer or bubbles in the tube. Lysis Buffer might cause the reverse transcription reaction to fail.
5. Remove the tube from the magnet, and pipet 1.0 mL of cold Bead Wash Buffer into the tube. Pipet-mix.
6. Place the tube on the 1.5-mL magnet for ≤2 minutes. Remove the supernatant.
7. Remove the tube from the magnet, and pipet 1.0 mL of cold Bead Wash Buffer into the tube. Pipet-mix, and place on ice.

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

   Note: If performing the TCR and/or BCR assay, stop here and proceed with respective protocol to continue cDNA synthesis.

Performing reverse transcription

1 Ensure that the SmartBlock™ Thermoblock for ThermoMixer® C is at 37 °C.

2 In the pre-amplification workspace, pipet the following reagents into a new 1.5-mL LoBind tube on ice:

cDNA mix

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

3 Gently vortex mix, briefly centrifuge, and place back on ice.

4 Place the tube of washed BD Rhapsody™ Enhanced Cell Capture Beads on the 1.5-mL tube magnet for ≤2 minutes. Remove the supernatant.

5 Remove the tube from the magnet and pipet 200 µL of cDNA mix into the beads. Pipet-mix.

Keep the prepared cDNA mix with beads 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 bead suspension on the SmartBlock™ Thermoblock for ThermoMixer® C at 1,200 rpm and 37 °C for 20 minutes.

Shaking is critical for this incubation.

8 Place the tube on ice.

Treating the sample with Exonuclease I

1 Set one thermomixer to 37 °C and a second thermomixer to 80 °C.

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

2 In the pre-amplification workspace, pipet the following reagents into a new 1.5-mL LoBind tube on ice:

Exonuclease I mix

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

3 Gently vortex mix, briefly centrifuge, and place back on ice.

4 Place the tube of BD Rhapsody™ Enhanced Cell Capture Beads with cDNA mix on the 1.5-mL tube magnet for ≤2 minutes. Remove the supernatant.

5 Remove the tube from the magnet, and pipet 200 µL of Exonuclease I mix into the tube. Pipet-mix.

6 Incubate the bead suspension on the thermomixer at 1,200 rpm and 37 °C for 30 minutes.

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

7 Incubate the bead suspension on the thermomixer (no shaking) at 80 °C for 20 minutes.

Do not exceed this inactivation temperature and incubation time.

8 Place the tube on ice for ~1 minute.

9 Place the tube on the magnet for ≤1 minute until clear. Remove the supernatant.

10 Remove the tube from the magnet, and pipet 200 µL of cold Bead Resuspension Buffer into the tube. Pipet-mix.

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

11 Proceed to library preparation. See the *Single-Cell Analysis Workflow with BD Rhapsody™ System*.

Troubleshooting

For additional troubleshooting, see the troubleshooting section in the instrument user guide.

For technical support, contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com.

Observation	Possible causes	Recommended solutions
No pellet after centrifuging cells or very few cells.	Rare or dilute sample.	After each centrifugation step, leave 50 µL of supernatant.

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