

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

For complete instrument procedures and safety information, and workflow, see the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide* (Doc ID: 214063).

Introduction

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

Required materials

- BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731)
- BD Rhapsody™ Cartridge Kit (Cat. No. 633733)
- BD Rhapsody™ cDNA Kit (Cat. No. 633773)
- Falcon® Tube with Cell Strainer Cap (Corning Cat. No. 352235)
- BD Rhapsody™ P1200M pipette (Cat. No. 633704)
- BD Rhapsody™ P5000M pipette (Cat. No. 633705)
- Large magnetic separation stand (V&P Scientific, Inc. Cat. No. VP 772FB-1)
- 15 mL tube adapter (V&P Scientific Cat. No. VP 772FB-1A)
- 6-Tube Magnetic Separation Rack for 1.5 mL tubes (New England Biolabs Cat. No. S1506S)

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

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.
- To ensure an air-tight seal with the BD Rhapsody™ P1200M (Cat. No. 633704) and P5000M (Cat. No. 633705) 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 (Cat. No. 633773) at room temperature (15°C to 25°C), and then place on ice. Keep enzymes at –25°C to –15°C.
- Place on ice:
 - Sample Buffer (Cat. No. 650000062)
 - 1 M DTT (Cat. No. 650000063)
 - Lysis Buffer (Cat. No. 650000064)
 - Cell Capture Beads (Cat. No. 650000089)
- 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 a heat block or additional thermomixer to 80°C.
- Prepare a single cell suspension. See *Preparing Single Cell Suspensions Protocol* (Doc ID: 210964).
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single Cell Suspensions Protocol* (Doc ID: 210964).

Priming and treating the BD Rhapsody Cartridge

Prime and treat the BD Rhapsody Cartridge (Cat. No. 400000847). For complete instructions, see instrument user guide.

Express instrument slider	Position
Front	WASTE
Side	0

Step no.	Reagent to load	Volume (µL)	P1200M pipette mode	Incubation at room temp.
1	100% ethyl alcohol	700	Prime/Treat	—
2	Air	700	Prime/Treat	—
3	Room temp. Cartridge Wash Buffer 1 (Cat. No. 650000060)	700	Prime/Treat	1 min
4	Air	700	Prime/Treat	—
5	Room temp. Cartridge Wash Buffer 1 (Cat. No. 650000060)	700	Prime/Treat	10 min
6	Air	700	Prime/Treat	—
7	Room temp. Cartridge Wash Buffer 2 (Cat. No. 650000061)	700	Prime/Treat	≤4 h

Counting and preparing a single cell suspension for cartridge loading

- 1 Treat 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

For detailed instructions, see instrument user guide.

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

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

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

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

$$V_n = 34 \mu\text{L} + 17 \mu\text{L} = 51 \mu\text{L}$$

- 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:

$$B = 650 \mu\text{L} - 51 \mu\text{L} = 599 \mu\text{L}$$

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

- 7 According to the calculations in steps 3–6, prepare the cell suspension in cold Sample Buffer (Cat. No. 650000062) in a new 1.5 mL LoBind Tube.

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

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

Loading cells in cartridge

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

Material to load	Volume (μL)	Pipette mode
Air	700	Prime/Treat
<ul style="list-style-type: none"> 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.

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

- 2 If necessary, wipe condensation from top cartridge surface for optimal scanning.
 3 Incubate at room temperature (15°C to 25°C) for 15 minutes. During 15 minute incubation, prepare Cell Capture Beads (Cat. No. 650000089).

Preparing Cell Capture Beads

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. Use low retention pipette tips and LoBind Tubes. Keep beads cold, and pipet-mix only.

- Place Cell Capture Bead tube on magnet for 1 minute, and remove storage buffer.
- Remove tube from magnet, and pipet 750 μL cold Sample Buffer (Cat. No. 650000062) into tube.
- Pipet-mix, and place on ice.

Loading and washing Cell Capture Beads

- 1 Set P1200M pipette to Prime/Treat mode.
- 2 Load cartridge with materials listed using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Prime/Treat
<ul style="list-style-type: none">• Set P1200M pipette to Bead Load mode.• Use a manual P1000 to gently pipet-mix beads in cold Sample Buffer (Cat. No. 650000062). Immediately load.		
Cell Capture Beads	630	Bead Load

- 3 Incubate the cartridge at room temperature (15°C to 25°C) for 3 minutes.
- 4 Place cartridge on the plate shaker plate adapter.
- 5 Shake the cartridge at room temperature (15°C to 25°C) for 15 seconds:
 - Eppendorf ThermoMixer® C: 1,000 rpm
 - Eppendorf MixMate®: 1,000 rpm OR
 - MicroPlate Genie®: 1,600 rpm. Set external timer to 15 seconds.
- 6 Blot outlet drip with lint-free wiper.
- 7 Return cartridge to Express instrument, and wait 30 seconds.
- 8 Set P1200M pipette to Wash mode.
- 9 Load cartridge with materials listed using the P1200M pipette:

Material to load	Volume (µL)	Pipette mode ^a
Air	700	Wash
Cold Sample Buffer (Cat. No. 650000062)	700	Wash
Air	700	Wash
Cold Sample Buffer (Cat. No. 650000062)	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 1 M DTT (Cat. No. 650000063) to one 15 mL Lysis Buffer bottle (Cat. No. 650000064). Check **Add DTT** box.

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

- 2 Briefly vortex lysis mix, place on ice.
- 3 Move the left slider to **LYSIS** on Express instrument.
- 4 Set P1200M pipette to Lysis mode.
- 5 Load cartridge with materials listed using the P1200M pipette:


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

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

Maintain recommended lysis time for best performance.

Retrieving Cell Capture Beads

- 1 Place the 5 mL LoBind Tube in Express instrument drawer.
- 2 Ensure P5000M pipette is set to **Retrieval** mode.
- 3 Move the front slider to **BEADS** on Express instrument.
- 4 Move the left slider to **RETRIEVAL**.
- 5 Leave Retrieval magnet in down position for 30 seconds.
- 6 Aspirate 5,000 μ L Lysis Buffer with DTT with the P5000M pipette.
- 7 Press down on P5000M pipette to seal against the gasket.
- 8 Move the left slider to the middle position (0), and *immediately* load 4,950 μ L Lysis Buffer with DTT.
- 9 Remove pipette from gasket, and purge tip.
- 10 Move the front slider to **OPEN**, and place the 5 mL LoBind Tube on large magnet with 15 mL tube adapter (V&P Scientific Cat. No. VP 772FB-1A) for 1 minute.
- 11 Immediately proceed to [Washing Cell Capture Beads](#).
- 12 Appropriately dispose of cartridge, Waste Collection Container, and Lysis Buffer with DTT.

	<p>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.</p>
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- 13 Clean Express instrument with 10% bleach or 70% ethyl alcohol.

Washing Cell Capture Beads

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

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

- 5 Remove tube from magnet and pipet 1.0 mL of cold Bead Wash Buffer (Cat. No. 650000065) into tube. Pipet-mix.
- 6 Place tube on 1.5 mL tube magnet for ≤ 2 minutes, and remove supernatant.
- 7 Remove tube from magnet, and pipet 1.0 mL cold Bead Wash Buffer (Cat. No. 650000065) into tube. Pipet-mix, and place on ice.

Start reverse transcription ≤ 30 minutes after washing retrieved Cell Capture Beads with Bead Wash Buffer.

Performing reverse transcription

- 1 Ensure that the SmartBlock Thermoblock for ThermoMixer C is at 37°C.
- 2 In pre-amplification workspace, pipet reagents in order into a new 1.5 mL LoBind Tube on ice:
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 mix, briefly centrifuge, and place back on ice.
- 4 Place tube of washed Cell Capture Beads on 1.5 mL tube magnet for ≤ 2 minutes. Remove supernatant.
- 5 Remove tube from magnet and pipet 200 µL cDNA mix into beads. Pipet-mix.

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

- 6 Transfer bead suspension to new 1.5 mL LoBind Tube.
- 7 Incubate bead suspension on SmartBlock Thermoblock for ThermoMixer C at 1,200 rpm and 37°C for 20 minutes. **Shaking is critical for this incubation.**
- 8 Place on ice.

Treating sample with Exonuclease I

- 1 Set one thermomixer to 37°C and a second thermomixer or heat block to 80°C.
- 2 In pre-amplification workspace, pipet reagents into a new 1.5 mL LoBind Tube on ice:
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

- 3 Gently vortex mix, briefly centrifuge, and place back on ice.
- 4 Place tube of Cell Capture Beads with cDNA mix on magnet for ≤ 2 minutes. Remove supernatant.
- 5 Remove tube from magnet, and pipet 200 µL Exonuclease I mix into tube. Pipet-mix.
- 6 Incubate bead suspension on thermomixer at 1,200 rpm and 37°C for 30 minutes.
- 7 Incubate bead suspension on thermomixer (no shaking) or heat block at 80°C for 20 minutes.

- 8 Place tube on ice for ~1 minute.
- 9 Place tube on magnet for ≤ 1 minute until clear. Remove supernatant.
- 10 Remove tube from magnet, and pipet 200 μL cold Bead Resuspension Buffer (Cat. No. 650000066) into tube. Pipet-mix.
Stopping point: Exonuclease I-treated beads can be stored at 2°C to 8°C for ≤ 3 months.
- 11 Proceed to library preparation. See the *Single Cell Analysis Workflow with BD Rhapsody™ Systems* (Doc ID: 220524).

For BD Biosciences technical support, contact researchapplications@bd.com, 1.877.232.8995, prompt 2, 2; 2350 Qume Drive, San Jose, CA 95131 USA

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