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

For complete instrument procedures and safety information, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID: 214062).

Introduction

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

Required materials

- Calcein AM (Thermo Fisher Scientific Cat. No. C1430)
- Draq7™ (Cat. No. 564904)
- 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)
- INCYTO™ disposable hemocytometer (INCYTO Cat. No. DHC-N01-5)
- 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 Calcein AM. Once at room temperature (15°C to 25°C), resuspend Calcein AM (1 mg; Thermo Fisher Scientific Cat. No. C1430) in 503.0 µL DMSO for a final stock concentration of 2 mM. Follow manufacturer's instructions, and protect from light.
- 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 BD Rhapsody Cartridge (Cat. No. 400000847). For detailed instructions, see instrument user guide.

Express instrument slider	Position
Front	**WASTE**
Side	**0**

Step no.	Material 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**

Staining cells with viability markers

Protect Calcein AM and Draq7 from light until ready to use.

1 If cells are not resuspended in cold Sample Buffer (Cat. No. 650000062), centrifuge cell suspension at 400 × *g* for 5 minutes, aspirate supernatant, and leave ~20 µL of residual supernatant. Add up to 620 µL total volume of cold Sample Buffer.

Performance might be impacted if samples are not in Sample Buffer. For rare samples that are not resuspended in Sample Buffer before cell loading, proceed at your own risk, or contact tech support.

2 Add 3.1 µL of 2 mM Calcein AM (Thermo Fisher Scientific Cat. No. C1430) and 3.1 µL of 0.3 mM Draq7 (Cat. No. 564904) to 620 µL cell suspension (1:200 dilution) in cold Sample Buffer (Cat. No. 650000062).

3 Gently pipet-mix.

4 Incubate at 37°C in dark for 5 minutes.

5 Filter cells through Falcon Tube with Cell Strainer Cap (Corning Cat. No. 352235).

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

6 Count cells immediately using the scanner. Gently pipet 10 µL into INCYTO disposable hemocytometer (INCYTO Cat. No. DHC-N01-5).

Keep remaining cells on ice, and protect from light.

Counting and preparing a single cell suspension for cartridge loading

For detailed instructions on counting cells with the BD Rhapsody™ Scanner, see instrument user guide.

1 Insert hemocytometer into Hemocytometer Adapter (Cat. No. 633703), and tap **Scan**.

2 Place adapter on scanner tray, and tap **Continue**.

3 Select protocol and select, or enter the experiment name, sample name, and user.

4 Tap **Side A** or **Side B** and **Start Side A Scan** or **Start Side B Scan** (**Cell Count**).

5 After the scan is complete, tap **OK**.

6 Tap **Scan**, and enter a new sample name to scan other side of the hemocytometer. Repeat steps 4–5, or tap **Eject**, and remove adapter. Tap **Done.**

7 Tap **Analysis** and experiment name to view **total cell concentration** and **cell viability**.

8 Proceed as follows:
- If cell concentration is ≤1,000 cells/µL, proceed to step 9.
- If the cell concentration is >1,000 cells/µL, dilute cell suspension in cold Sample Buffer (Cat. No. 650000062) to ~200–800 cells/µL. Repeat steps 1–7, and then step 9.

9 Tap **Prepare** to display Samples Calculator screen.

10 Dispose of the hemocytometer.

Minimize the time between cell pooling and single cell capture.

11 Use the Samples Calculator to obtain stock cell and buffer volumes from the scanner to prepare a cell suspension of 650 µL. See instrument user guide.

12 Prepare the cell suspension according to the displayed volumes on the scanner.

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

13 If samples were not filtered before counting cells, filter through a 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**
• Set P1200M pipette to **Cell Load** mode. • Pipet-mix 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. To incubate the cartridge on the scanner, enter time delay of 15 minutes before tapping **Start Cell Load Scan** (**Cell Load** step).

4 During 15 minute incubation, prepare Cell Capture Beads (**Cat. No. 650000089**). See Preparing Cell Capture Beads.

5 Image the cells in the cartridge. Perform scanner step: **Cell load**. See instrument user guide.

6 After the scan is complete, tap **OK** and **Eject**. Remove cartridge and tap **Scan** or **Done**. After scan, confirm analysis is running.

Preparing Cell Capture Beads

Keep 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.

1 Place Cell Capture Bead tube on magnet for 1 minute, and remove storage buffer.

2 Remove tube from magnet, and pipet 750 µL cold Sample Buffer (Cat. No. 650000062) into tube.

3 Pipet-mix, and place on ice.

4 After Cell Load scan and analysis is running, proceed to Loading and washing Cell Capture Beads.

Loading and washing Cell Capture Beads

1 Place cartridge on Express instrument.

2 Set P1200M pipette to **Prime/Treat** mode.

3 Load cartridge with 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 beads in cold Sample Buffer (Cat. No. 650000062). Immediately load.		
Cell Capture Beads	630	**Bead Load**

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

5 Perform scanner step: **Bead Load**.

6 Place cartridge on Express instrument.

7 Set P1200M pipette to **Wash** mode.

8 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.

9 Perform scanner step: **Bead Wash**.

Lysing cells

Avoid bubbles.

1 Add 75.0 µL of 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, and place on ice.

3 Place cartridge on Express instrument.

4 Move the left slider to **LYSIS**.

5 Set P1200M pipette to **Lysis** mode.

6 Load cartridge with materials listed using the P1200M pipette:

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

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

Maintain the recommended lysis time for best performance.

Retrieving Cell Capture Beads

1 Place the 5 mL LoBind Tube in Express instrument drawer.

2 Ensure that the 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 µL 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 µL 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 During the 1 minute incubation, perform scanner step: **Retrieval**.

12 Immediately proceed to Washing Cell Capture Beads.

13 Appropriately dispose of 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.

14 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 1.5 mL magnet for ≤2 minutes. 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 cold Bead Wash Buffer (Cat. No. 650000065) into tube. Pipet-mix.

6 Place tube on 1.5 mL magnet for ≤2 minutes. 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 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 a 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.**

During reverse transcription incubation, view image analysis to see if analysis metrics passed.

8 Place on ice.

Treating the 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 1.5 mL tube 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).

Troubleshooting

Observation	Possible causes	Recommended solutions
Reported viability from the BD Rhapsody™ Scanner suspected to be too high	Draq7 staining in the current protocol is optimized for cell lines. The optimal concentration of Draq7 might be higher.	Before the BD Rhapsody experiment, optimize the Draq7 concentration for your cell types according to the manufacturer's protocol. See bdbiosciences.com/ds/pm/tds/564904.pdf.
No pellet after centrifuging cells or very few cells	Rare or dilute sample	After each centrifugation step, leave 50 µL of supernatant.

For additional troubleshooting on scanning or cartridge loading, see troubleshooting in instrument user guide.

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