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

For Research Use Only

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Regulatory Information

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

History

Revision	Date	Change made
210966 Rev. 1.0	2018-07	Initial release
23-22951-00 (210966 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
23-22951(01) (210966 Rev. 3.0)	2022-01	Added Enhanced Cell Capture Beads and part numbers.

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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.

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

Required materials

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

- Calcein AM (Thermo Fisher Scientific Cat. No. C1430)
- DRAQ7™ (BD Pharmingen™ Cat. No. 564904)
- BD Rhapsody™ Targeted mRNA and AbSeq Reagent Kit (Cat. No. 633771), which contains the following components:
  - BD Rhapsody™ Cartridge Reagent Kit (Cat. No. 633731) or BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. No. 664887)
  - 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)

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 pipette 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 the 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 the following components of the BD Rhapsody™ Cartridge Reagent Kit:
  - Sample Buffer (Cat. No. 650000062)
  - 1 M DTT (Cat. No. 650000063)
  - Lysis Buffer (Cat. No. 650000064)
  - Cell Capture Beads (Cat. No. 650000089) or BD Rhapsody™ Enhanced Cell Capture Beads (Cat. No. 700027881)
- 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.
- 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 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 (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 hr

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 the cell suspension at 400 × *g* for 5 minutes, aspirate the 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 of cell suspension (1:200 dilution) in cold Sample Buffer (Cat. No. 650000062).

3 Gently pipet-mix.

4 Incubate at 37°C in the 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. We recommend filtering the final sample before loading cells into the cartridge.

6 Count cells immediately using the scanner.

a Ensure cells are well suspended by gently pipet-mixing.

b Pipet 10 µL into the INCYTO™ disposable hemocytometer (INCYTO Cat. No. DHC-N01-5).

Keep the 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 the instrument user guide.

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

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

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

4 Tap **Side A** or **Side B**, then **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 the other side of the hemocytometer. Repeat steps 4–5, or tap **Eject** and remove the adapter. Tap **Done.**

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

8 Proceed as follows:

- If the cell concentration is ≤1,000 cells/µL, proceed to step 9.
- If the cell concentration is >1,000 cells/µL, dilute the 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 the 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 the instrument user guide.

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

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

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

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.

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°C to 25°C) for 15 minutes. To incubate the cartridge on the scanner, enter a time delay of 15 minutes before tapping **Start Cell Load Scan** (Cell Load step).

4 During the 15-minute incubation, prepare the Cell Capture Beads (Cat. No. 650000089) or BD Rhapsody™ Enhanced Cell Capture Beads (Cat. No. 700027881). See Preparing Cell Capture Beads in the following section.

5 Image the cells in the cartridge. Perform the scanner step: Cell Load. For more information, see the instrument user guide.

6 After the scan is complete, tap **OK** and **Eject**. Remove the cartridge and tap **Scan** or **Done**. After the scan, confirm the 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 the beads cold, and pipet-mix only.

1 Place the Cell Capture Bead 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 (Cat. No. 650000062) into the tube.

3 Pipet-mix, and place on ice.

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

Loading and washing Cell Capture Beads

1 Place the cartridge on the Express instrument.

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

3 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 (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 the scanner step: Bead Load.

6 Place the cartridge on the Express instrument.

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

8 Load the cartridge with the 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 the 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 the lysis mix, and place on ice.

3. Place the cartridge on the Express instrument.

4. Move the left slider to LYSIS.

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

6. Load the cartridge with the 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 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 (V&P Scientific Cat. No. VP 772FB-1A) for 1 minute.

11 During the 1-minute incubation, perform the scanner step: Retrieval.

12 Immediately proceed to Washing Cell Capture Beads in the following section.

13 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.

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

Washing Cell Capture Beads

1 After the 1-minute incubation, leaving the 5-mL tube containing retrieved 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 (Cat. No. 650000065) 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 (Cat. No. 650000065) into the tube. Pipet-mix, and place on ice.

Start reverse transcription ≤30 minutes after washing the 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 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 (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. 700026321))	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 the tube of washed 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 During reverse transcription incubation, view the image analysis to see if the analysis metrics passed.

9 Place 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 (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 the tube of 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 (Cat. No. 650000066) into the tube. Pipet-mix.

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

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

Troubleshooting

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

For technical support, contact scomix@bdscomix.bd.com.

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 the DRAQ7™ technical data sheet, 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.