BD Rhapsody™ HT Xpress System

Single-Cell Capture and cDNA Synthesis

Protocol

23-24253(02)
2024-01

For Research Use Only

Regulatory information

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

History

Revision	Date	Change Made
23-24253(01)	2023-03	Initial version.
23-24253(02)	2024-01	Added minor text corrections. Added note detailing storage temperature and duration to BD Rhapsody™8-lane cartridge storage procedure.

Contents

Introduction

This protocol describes cell loading in the BD Rhapsody™ 8-Lane Cartridge and single-cell capture with the BD Rhapsody™ HT Xpress System.

For complete instrument procedures and safety information, see the *BD Rhapsody™ HT Xpress 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™ Enhanced Cartridge Reagent Kit v3	BD Biosciences	667052
BD Rhapsody™ 8-Lane Cartridge	BD Biosciences	666262
BD Rhapsody™ cDNA Kit	BD Biosciences	633773
BD Rhapsody™ Dual Index Kit	BD Biosciences	667237
Absolute ethyl alcohol, molecule biology grade	Major supplier	–
Nuclease-free water	Major supplier	–
Trypan blue stain	Major supplier	–
Cell viability stain	Major supplier	–
Dimethyl sulfoxide (DMSO)	Major supplier	–
70% ethyl alcohol or 70% isopropyl alcohol[a]	Major supplier	–
a. To clean the BD Rhapsody™ HT Xpress System and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide*. Instead of 70% alcohol, 10% (v/v) bleach can be used.		
For additional indexing primers for high-throughput library preparation workflows, the BD Rhapsody™ Dual Index Kit is required.		

Recommended consumables

Material	Supplier	Catalog no.
Gilson™ PIPETMAN™ DIAMOND Tipack™ Filter Tips, 100-1200 µL for BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) (Recommended) Or, ZAP™ SLIK 1000 µL Low Retention Aerosol Filter Pipet Tips for BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) (Alternative)	Thermo Fisher Scientific Labcon	F171803 or F171803G 1177-965-008-9
60 mL reagent reservoir self-standing[a]	BD Biosciences	666626
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 10 mL	VistaLab	3054-1012 3054-1013
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 25 mL	VistaLab	3054-1002 3054-1003
Falcon® tube with cell strainer cap	Corning	352235
Corning® 96-well polypropylene cluster tube, 8-tube strip format, sterile[b]	Corning	4413
DNA LoBind® tubes, 1.5-mL	Eppendorf	30108051
DNA LoBind® tubes, 2.0-mL	Eppendorf	022431048
Low-retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
INCYTO™ disposable hemocytometer	INCYTO	DHC-N01-5
Deep 96-Well 2 mL Polypropylene plate	Major supplier	–
Pre-moistened cleaning wipes with 70% ethyl alcohol or 70% isopropyl alcohol.	Major supplier	–
Lint-free wipes	Major supplier	–

a. Waste collection container for the BD Rhapsody™ HT Xpress System.

b. These are the bead retrieval tubes to be used with the BD Rhapsody™ HT Xpress System.

Required equipment

Material	Supplier	Catalog no.
BD Rhapsody™ HT Xpress Package[a]	BD Biosciences	700036499
BD Rhapsody™ P8xP1200µL Pipette - HTX[b]	BD Biosciences	500066280
BD Rhapsody™ P1200µL Pipette - HTX	BD Biosciences	500066148
Cell counter	Major Supplier	–
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Centrifuge and rotor with adapters for 5-mL 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
Incubator at 37 °C	Major supplier	–
Pipettes (P10, P20, P200, P1000)	Major supplier	–
Multi-channel pipette (P200)	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. Includes BD Rhapsody™ P8x1200uL Pipette - HTX and Hamilton 60mL Waste Reservoirs.
b. Part of the BD Rhapsody™HT Single-Cell Analysis Package. Items can be ordered separately.

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

Guidelines for good pipetting

The following guidelines apply to the BD Rhapsody™ P8xP1200µL Pipette and BD Rhapsody™ P1200µL Pipette:

- BD Rhapsody™ P8xP1200µL Pipette: Push the tip holder into the tips while rocking back and forth to ensure a firm and airtight seal.
- BD Rhapsody™ P1200µL Pipette: Push the tip holder into the tip using a slight twisting motion to ensure a firm and airtight seal.
- Hold the pipette vertically and immerse the tip 2-4 mm in the liquid. Make sure that the reservoir has enough volume to avoid aspirating air.
- Press the push button to aspirate the set volume of liquid. Wait for 2-3 seconds. Then withdraw the pipette tip from the liquid. Ensure that every tip aspirates the same volume of the liquid before loading in the cartridge.
- While removing pipette from the reservoir, draw the tip along the inside surface of the vessel.
- When dispensing, ensure that the pipette tips are seated perpendicular to the inlet of the BD Rhapsody™ 8-Lane Cartridge.
- Press the push button to dispense. While tips are still inserted in the cartridge inlet, wait for at least a few seconds before releasing the push button to expel any residual liquid from the tip.

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
  - Bead Wash Buffer
- 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 4 or 8 lanes at the same time, after opening the DTT tube once, seal and store the tube at –20 °C. Otherwise, 1 M DTT can be aliquoted for single use and stored at –20 °C.
- Aliquot 100% ethyl alcohol and cartridge reagent buffers in 10-mL or 25-mL reagent reservoirs as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
100% ethyl alcohol	0.05	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cartridge Wash Buffer 1	0.76	3.50	5.25	7.00	8.75	10.50	12.25	14.00
Cartridge Wash Buffer 2	0.38	2.00	3.00	4.00	5.00	6.00	7.00	8.00

Priming and treating BD Rhapsody™ 8-Lane Cartridge

Prime and treat the BD Rhapsody™ 8-Lane Cartridge. For detailed instructions, see the instrument user guide.

- Keep the foil pouch and desiccant to store partially used cartridge.
- Place waste collection container and Cluster Tube in the BD Rhapsody™ HT Xpress System.
- Carefully peel off the seal on the cartridge inlet depending on the number of lanes to be used.
- Refer to the following table for instrument slider position.

BD Rhapsody™ HT Xpress System slider	Position
Front Slider	Waste
Retrieval Slider	Inactive (Back)

Notes:

- Ethyl alcohol priming of the cartridge followed by air purge provides full coverage of the array during the Prime/Wash step (Step 2 on the following table).
- Random bubbles (<3 mm diameter in size) that occur during the Priming steps does not affect cartridge performance.
- In lanes where bubbles >3 mm in size are observed, aspirate and dispense air using the Prime/Wash mode and repeat Step 1 with 100% ethyl alcohol. Only do this in the Priming steps.
- Uneven fluidic front observed on different lanes does not affect cartridge performance.
- Discard used tips even if residual volume is observed after dispensing.
- It is recommended to use a P20 pipette to aspirate buffer pooling at the inlet. Aspirate at an angle to avoid accidental aspiration of buffer volume in the microwell array. Only do this in the Priming steps.

Step no.	Material to load	Volume (µL) 1 Lane	Pipette Mode	Incubation at room temperature
1	100% ethyl alcohol	50	EtOH Prime	—
2	Air	380	Prime/Wash	—
3	Room temp. Cartridge Wash Buffer 1	380	Prime/Wash	1 min
4	Air	380	Prime/Wash	—
5	Room temp. Cartridge Wash Buffer 1	380	Prime/Wash	3 min
6	Air	380	Prime/Wash	—
7	Room temp. Cartridge Wash Buffer 2	380	Prime/Wash	≤4 hr

Counting and preparing 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. Filter cells through Falcon® Tube with Cell Strainer Cap.

   Note: For low-abundance or low-volume samples, filtering is optional at this step.
3. Determine the desired number of cells to capture in the BD Rhapsody™ 8-Lane Cartridge. See the *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-free workflow* for a table containing estimated multiplet rates based on the number of captured cells on retrieved BD Rhapsody™ Enhanced Cell Capture Beads.
4. Determine the pooling ratio of samples to load onto the BD Rhapsody™ 8-Lane 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.
5. 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™ 8-Lane 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

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

   V_n = 34 µL + 17 µL= 51 µL

7. Calculate the volume of cold Sample Buffer, *B*, that is needed to bring the final volume of cell suspension to 380 µL. Using the example in **step 5**:

   ***B* = 380 µL – 51 µL= 329 µL**

8. 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 .

9. Transfer each tube of prepared cells to a 96-deep well plate for multiple lane loading. Keep on ice.

   Minimize the time between cell pooling and single-cell capture.

Loading cells in BD Rhapsody™ 8-Lane Cartridge

1 Load the cartridge with materials listed using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette):

Material to load	Volume (µL) 1 Lane	Pipette mode
Air	380	Prime/Wash
• Gently pipet mix with a multi-channel pipette to completely resuspend the cells. • Set the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to **Load** mode. • Immediately load.		
Cell suspension	320	**Cell Load**

Note: Air bubbles that might appear at the inlet or outlet of the cartridge (outside of the microwell array) do not affect cartridge performance.

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

3 Incubate at room temperature (15–25 °C) for 8 minutes.

During the 8-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. Remove and discard the storage buffer.
2. Remove the tube from the magnet, and pipet 380 µL of cold Sample Buffer into the tube.
3. Pipet-mix, and place on ice.
4. Transfer each tube of the BD Rhapsody™ Enhanced Cell Capture Beads to a 96-deep well plate for multiple lane loading. Keep on ice until use.
5. Proceed to Loading and washing BD Rhapsody™ Enhanced Cell Capture Beads in the following section.

Loading and washing BD Rhapsody™ Enhanced Cell Capture Beads

1 Place the cartridge on the BD Rhapsody™ HT Xpress System.

2 Load the cartridge with the materials listed using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette):

Material to load	Volume (µL) 1 Lane	Pipette mode
Air	380	Prime/Wash
• Set the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to **Mix** mode, gently mix cell capture beads by pressing the push button six times (waiting for aspiration or dispense to complete each time) or until the beads are completely resuspended. Make sure that the pipette tips are reaching the bottom of the wells to mitigate the chance of introducing air bubbles. Discard used pipette tips. • With a new set of pipette tips, set the pipette to **Load** mode. • Immediately load. Check the pipette tips to make sure that there are no air bubbles inside the tips before loading. Otherwise, dispense in the 96-deep well plate and aspirate with a new set of pipette tips.		
BD Rhapsody™ Enhanced Cell Capture Beads	320	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 BD Rhapsody™ HT Xpress System, and wait 30 seconds.

8 Aliquot Sample Buffer in 10 mL reagent reservoir as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
Sample Buffer	0.38	2.00	2.80	3.60	4.30	5.10	5.90	6.60

9 Set the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to **Prime/Wash** mode.

10 Load each lane used in the cartridge with the following materials using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette).

Material to load	Volume (µL) 1 Lane	Pipette mode
Air	380	Prime/Wash
Cold Sample Buffer	380	Prime/Wash
Air	380	Prime/Wash
Cold Sample Buffer	380	Prime/Wash

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 with 24 hours, and then discard.

2 Briefly vortex the lysis mix, and aliquot in the 10-mL or 25-mL reagent reservoir as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table. Place on ice.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
Lysis Buffer	0.28 / 1.0	3.75	5.60	7.50	9.40	11.25	13.10	15.00

3 Place the cartridge on the BD Rhapsody™ HT Xpress System.

4 Set the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to **Lysis** mode.

5 Load the cartridge with the materials listed using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette):

Material to load	Volume (µL)	Pipette mode
Lysis Buffer with DTT	280	Lysis

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

Maintain the recommended lysis time for best performance.

Note: Before retrieval, remove extra buffer that has pooled at the inlet with a P20 pipette to minimize overflow. Aspirate at an angle to avoid accidental aspiration of buffer volume in the microwell array.

Retrieving BD Rhapsody™ Enhanced Cell Capture Beads

1. Place the Cluster Tube, 8-tube strip format in the BD Rhapsody™ HT Xpress System drawer. Label the tubes appropriately.
2. Move the front slider to **BEADS** on the BD Rhapsody™ HT Xpress System.
3. Gently pull the top RETRIEVAL slider toward and on top of the BD Rhapsody™ 8-Lane Cartridge (ACTIVE). Make sure that the Retrieval magnet is in contact with the BD Rhapsody™ 8-Lane Cartridge.
4. Leave the Retrieval magnet in the ACTIVE position for 1 minute.
5. Set the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to **Retrieval** mode.
6. Aspirate 1,000 µL of Lysis Buffer with DTT using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette).
7. Press down on the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette) to seal against the gasket.
8. Push back the top RETRIEVAL magnet (INACTIVE), and immediately load 1,000 µL of Lysis Buffer with DTT.
9. Remove the pipette from the gasket, and discard the tip.
10. Move the front slider to **OPEN**, and remove the Cluster Tube with the bottom adapter to a flat, secure surface.
11. Move the front slider to **WASTE**. Do not throw away the waste container.
12. Blot the outlet drip on the bottom of the cartridge with a lint-free wipe to remove residual liquid.
13. Immediately proceed to Washing BD Rhapsody™ Enhanced Cell Capture Beads in the following section.
14. Keep the partially used cartridge on a flat surface while performing Washing BD Rhapsody™ Enhanced Cell Capture Beads and Performing Reverse Transcription.
15. Perform Washing used lanes and BD Rhapsody™ 8-Lane Cartridge storage procedure on page 21 during reverse transcription incubation.

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.

Washing BD Rhapsody™ Enhanced Cell Capture Beads

1 Remove the Cluster Tube from the bottom adapter. Gently pipet-mix the beads and transfer them to a new 1.5-mL LoBind tube. Keep on ice.

2 If there are still beads left in the Cluster Tube, add 100 µL of Lysis Buffer with DTT, rinse the Cluster Tube, and transfer to the 1.5-mL LoBind tube from the previous step.

3 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 may cause the reverse transcription reaction to fail.

4 Remove the tube from the magnet, and pipet 1.0 mL of cold Bead Wash Buffer into the tube. Pipet-mix.

   Note: When multiple samples are being processed, it is advised to keep the tubes on ice when they are not being washed.

5 Place the tube on the 1.5-mL magnet for ≤2 minutes. Remove the supernatant.

6 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 or 2.0-mL LoBind tube on ice:

cDNA mix

Component	For 1 lane (µL)	For 1 lane + 20% overage (µL)	For 4 lanes + 20% overage (µL)	For 8 lanes + 20% overage (µL)
RT Buffer	40.0	48.0	192.0	384.0
dNTP	20.0	24.0	96.0	192.0
RT 0.1 M DTT	10.0	12.0	48.0	98.0
Bead RT/PCR Enhancer	12.0	14.4	57.6	115.2
RNase Inhibitor	10.0	12.0	48.0	96.0
Reverse Transcriptase	10.0	12.0	48.0	96.0
Nuclease-Free Water	98.0	117.6	470.4	940.8
Total	**200.0**	**240.0**	**960.0**	**1,920.0**

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 and discard 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.

Note: Shaking is critical for this incubation.

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

9 Place the tube on ice.

Treating 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 or 2.0-mL LoBind tube on ice:

Exonuclease I mix

Component	For 1 lane (µL)	For 1 lane + 20% overage (µL)	For 4 lanes + 20% overage (µL)	For 8 lanes + 20% overage (µL)
10X Exonuclease I Buffer	20.0	24.0	96.0	192.0
Exonuclease I	10.0	12.0	48.0	96.0
Nuclease-Free Water	170.0	204.0	816.0	1,632.0
Total	**200.0**	**240.0**	**960.0**	**1,920.0**

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 and discard 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. Briefly centrifuge.

9 Place the tube on the magnet for ≤1 minute until clear. Remove and discard 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*.

Washing used lanes and BD Rhapsody™ 8-Lane Cartridge storage procedure

1 Move the front slider to **WASTE** on the BD Rhapsody™ HT Xpress System.

2 Aliquot nuclease-free water and 100% ethyl alcohol in a 10-mL reagent reservoir as follows depending on the number of lanes used. Do not aliquot for single lane. See the following table.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
Nuclease-free water	0.38	2.00	2.00	2.50	2.50	3.00	3.50	4.00
100% ethyl alcohol	0.05	2.00	2.00	2.00	2.00	2.00	2.00	2.00

3 Load each lane used in the cartridge with the materials listed using the BD Rhapsody™ P8xP1200µL Pipette (or BD Rhapsody™ P1200µL Pipette).

Material to load	Volume (µL)	Pipette mode	Incubation at room temperature
Air	380	Prime/Wash	—
Nuclease-free water	380	Prime/Wash	1 min
Air	380	Prime/Wash	—
100% ethyl alcohol	50	EtOH Prime	—
Air	380	Prime/Wash	—

4 Use a lint free wipe to remove liquid residue on the outside of the cartridge. Liquid residue inside the cartridge will not affect performance of the un-used lanes.

5 Make sure the seal of the un-used lane(s) are intact, place the cartridge for storage in the pouch provided with a desiccant bag, seal the double zipper bag, keep the cartridge flat, and store at room temperature in the dark.

6 Clean the BD Rhapsody™ HT Xpress System with 10% bleach or 70% ethyl alcohol.

7 Appropriately dispose of the waste collection container, unused cartridge buffers, and cartridge if all eight lanes have been used.

Note: Partially used cartridges can be stored up to 6 months at room temperature.

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.
Cannot move the RETRIEVAL slider.	The front slider is in the WASTE position.	Slide the front slider to the **BEADS** position.

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