Rhapsody™ System

Single-Cell ATAC-Seq BD OMICS-One™ WTA Next and Sample Tag

Library Preparation Protocol

23-25007(02)
2026-09

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Regulatory information

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History

Revision	Date	Change made
23-25007(01)	2025-10	Initial release.
23-25007(02)	2026-09	Updated with corrections for customer-impacted issues.

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Introduction

This protocol provides instructions on sample multiplexing and generating a single-cell ATAC library and whole transcriptome analysis (WTA) mRNA library with cell-capture beads using the BD Rhapsody™ Single-Cell Analysis System.

The BD® Nuclear Sample Multiplexing Kit utilizes an innovative antibody-oligo technology to provide higher sample throughput for single-nucleus library preparation. Every antibody-oligo in the BD® Nuclear Sample Multiplexing Kit, referred to as a Sample tag, has a unique sample oligo barcode conjugated to an antibody that can universally target nuclei. Up to six samples can be labeled and pooled prior to single-cell capture with the BD Rhapsody Single-Cell Analysis System.

To generate a single-cell ATAC library with the BD Rhapsody™ Single Cell Analysis System, a specific variant of Tn5 transposase is used to fragment and simultaneously insert Next Generation Sequencing (NGS) primers into the accessible chromatin regions of individual cells or nuclei for sequencing on compatible Illumina® sequencers. In combination with the BD OMICS-One™ WTA Next Amplification Kit, a 3' WTA approach is used to simultaneously profile mRNA expression of the tagmented single nuclei/cells. It enables uncovering insights into the chromatin structure and factors that affect gene expression of cells at single-cell level.

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

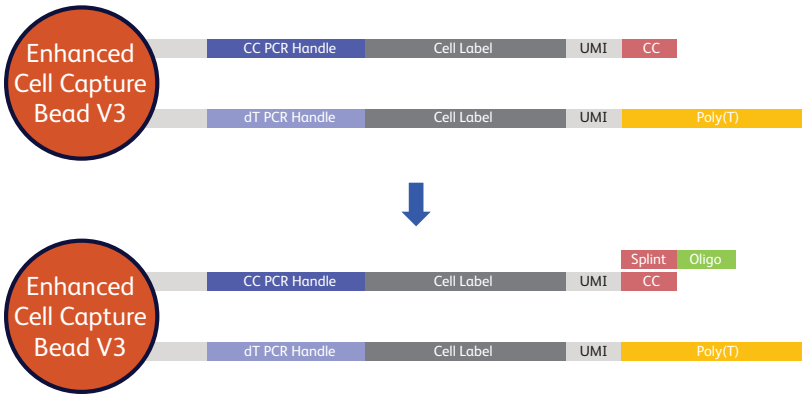
Workflow overview

The *BD Rhapsody™ System Single-Cell ATAC-Seq and BD OMICS-One™ WTA Next Library Preparation Protocol* offers a comprehensive multiomic solution, enabling simultaneous profiling of the epigenomic landscape and gene expression within the same single nuclei.

In the following diagrams, CC is an abbreviation for Custom Capture.

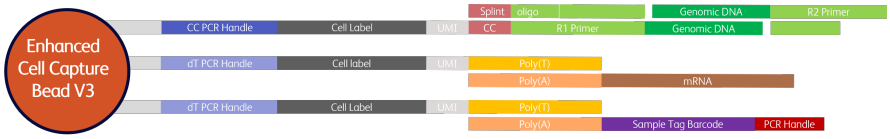
ATAC workflow

Splint bead: Starting from the BD Rhapsody™ Enhanced Bead V3 layout, add splint oligonucleotide to the beads to assist capturing of genomic DNA.

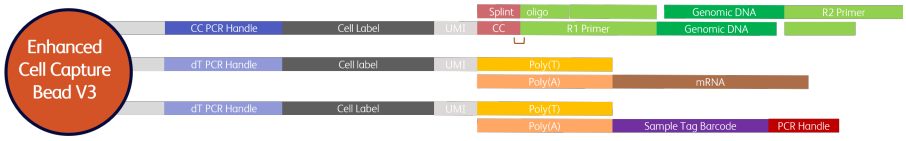


Nuclei preparation: Nuclei isolation protocol depends on the sample type. For details, see Nuclei preparation (page 23).
Tagmentation: During the bulk in-situ tagmentation process, expose the nuclei to a tagmentation mix, containing Tagmentase. This enzyme targets accessible genomic regions (open chromatin areas), cutting the DNA and simultaneously attaching preloaded adapter sequences to the ends of each DNA fragment. For substep details, see Tagmentation (page 27).

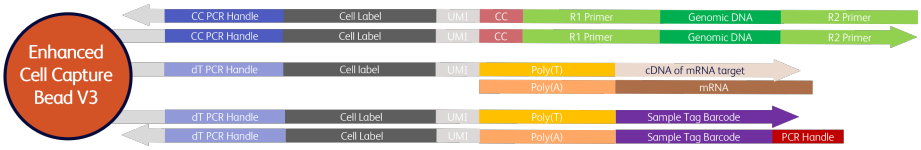
Single-cell capture: Perform cell lysis in a microwell. The genomic DNA sequences are captured by the splint-oligo-bonded CC strands. Then mRNA and Sample Tags are captured by poly(T).



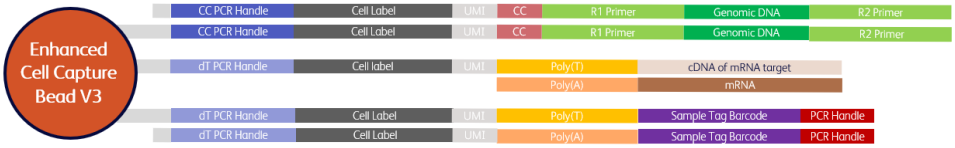
Ligation: Ligate BD Rhapsody™ bead oligo and tagmented DNA using DNA ligase.



Reverse transcription: This process performs ATAC fragment gap filling and extension to beads oligo. Complementary DNA and second strand of Sample Tag are synthesized from captured mRNA and sample tags.

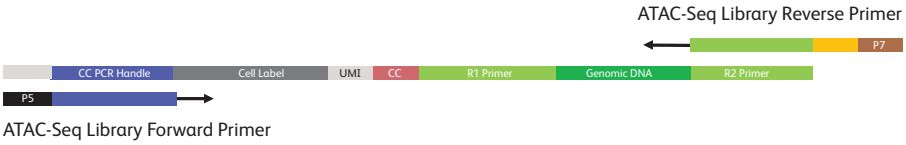


Splint oligo removal and Exonuclease I treatment: To remove unused oligos from the beads.



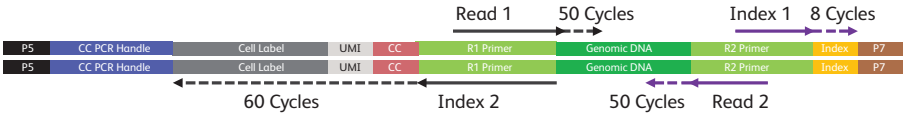
ATAC fragment denaturation and PCR amplification:

Supernatant: Denature the genomic DNA template off the bead. Illumina® adapters and indices are added during the ATAC product amplification.



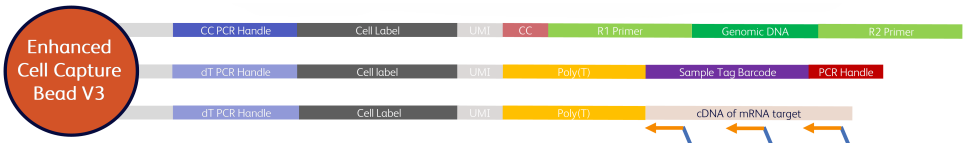
Bead: Proceed through the WTA workflow—see WTA library amplification workflow (page 8).

Sequencing:
Read 1: 50 cycles
Read 2: 50 cycles
Index 1: 8 cycles
Index 2: 60 cycles

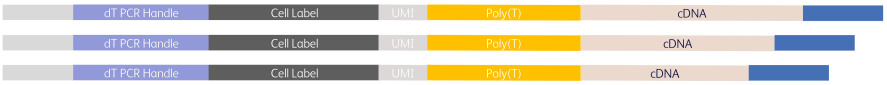


WTA library amplification workflow

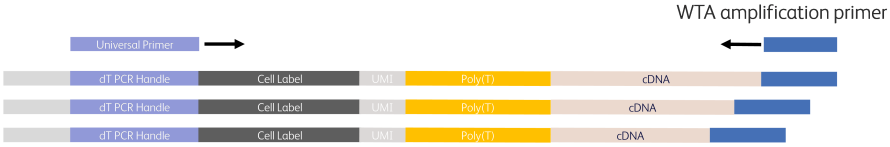
WTA Random Priming and Extension: Random priming on the bead.



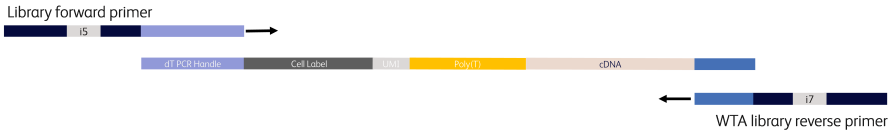
Denature off the RPE product



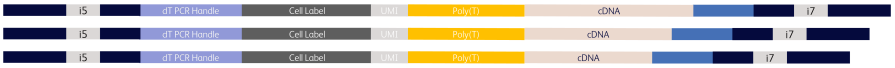
WTA RPE PCR: Amplify the RPE product.



WTA Index PCR: Add Illumina® adapters and indices.

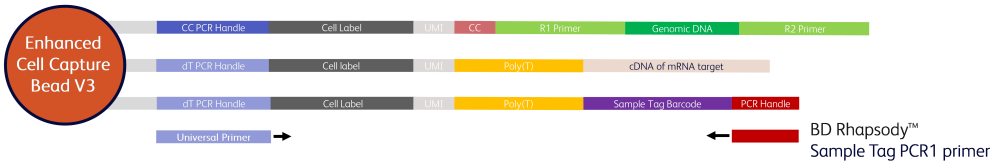


Final WTA library Product



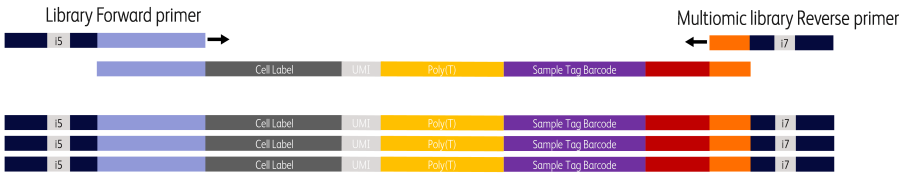
Sample Tag library amplification workflow

Sample Tag PCR1: Sample Tag product is amplified in PCR1.



Sample Tag PCR2: Sample Tag product is further amplified in PCR2.

Sample Tag Index PCR: Add Illumina adapters and indices.



Final Sample Tag library product:

Required and recommended materials

Required reagents

Store the reagents at the storage temperature specified on the label.

Material	Supplier	Catalog No.
BD Rhapsody™ Tagmentation and Supplemental Reagents Kit	BD Biosciences	571201
BD Rhapsody™ Multiomic ATAC-Seq Amplification Kit	BD Biosciences	571361
BD Rhapsody™ Enhanced Cartridge Reagent Kit V3[a]	BD Biosciences	667052
BD Rhapsody™ cDNA Kit	BD Biosciences	633773
BD OMICS-One™ WTA Next Amplification Kit	BD Biosciences	572620
BD OMICS-One™ Nuclei Buffer	BD Biosciences	572808
BD Rhapsody™ 8-Lane Cartridge	BD Biosciences	666262
AMPure® XP magnetic beads	Beckman Coulter	A63880
100% ethyl alcohol	Major supplier	–
Nuclease-free water	Major supplier	–
N,N-Dimethylformamide (DMF)	MilliporeSigma	D4551-250ML
DyeCycle™ Green[b]	Thermo Fisher Scientific	V35004
BD Pharmingen™ DRAQ7™[b]	BD Biosciences	564904
Tween 20	Sigma-Aldrich	P9416
Dimethylsulfoxide (DMSO)	Major supplier	–
BD® RNase Inhibitor (sold separately)	BD Biosciences	570751
70% ethyl alcohol or 70% isopropyl alcohol[c]	Major supplier	–
Ethylenediaminetetraacetic acid (EDTA)	Major supplier	–
MS Nucleoporin P62 sample tag 1	BD Biosciences	460291
MS Nucleoporin P62 sample tag 5	BD Biosciences	460293
MS Nucleoporin P62 sample tag 7	BD Biosciences	460294
MS Nucleoporin P62 sample tag 8	BD Biosciences	460295
MS Nucleoporin P62 sample tag 9	BD Biosciences	460296
MS Nucleoporin P62 sample tag 10	BD Biosciences	460297

Material	Supplier	Catalog No.
[a] The Enhanced Cartridge Reagent Kit V3 must be used to perform this protocol.		
[b] Either DyeCycle™ Green or DRAQ7™ is required (not both). Protect the dye from light. See manufacturer's storage recommendations.		
[c] To clean the BD Rhapsody™ Xpress System and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide*. Instead of 70% alcohol, 10% (w/v) bleach can be used.		

Recommended consumables

Supplies	Supplier	Catalog No.
Gilson™ PIPETMAN™ DIAMOND Tipack™ filter tips, 100–1200 µL for BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) (Recommended) Or ZAP™ SLIK 1000 µL low-retention aerosol filter pipet tips for BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) (Alternative)	Thermo Fisher Scientific Labcon	F171803G 1177-965-008-9
Low retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
Falcon® tube with cell strainer cap	Corning	352235
INCYTO disposable hemocytometer	INCYTO	CN DHC-N01-5
60-mL reagent reservoir self-standing[a]	BD Biosciences	666626
Corning® 96-well polypropylene cluster tube, 8-tube strip format, sterile[b]	Corning	4413
0.2-mL PCR 8-strip tubes	Major supplier	–
15-mL conical tube	Major supplier	–
50-mL conical tube	Major supplier	–
DNA LoBind® tubes, 1.5 mL	Eppendorf	022431021
DNA LoBind® tubes, 2.0 mL	Eppendorf	022431048
DNA LoBind® tubes, 5.0 mL	Eppendorf	0030108310
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
Deep 96-well 2-mL polypropylene plate	Major supplier	–
Lint-free cloth (Kim-Wipes)	Major supplier	–
Qubit™ assay tubes	Thermo Fisher Scientific	Q32856
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851
Agilent High Sensitivity DNA Kit	Agilent	5067-4626

[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.

Equipment

Equipment	Supplier	Catalog No.
Single-channel pipettes (P20, P200, P1000)	Major supplier	–
BD Rhapsody™ HT Xpress Package	BD Biosciences	666730
BD Rhapsody™ Scanner	BD Biosciences	633701
Hemocytometer adapter[a]	BD Biosciences	633703
BD Rhapsody™ P8xP1200 µL pipette-HTX[b]	BD Biosciences	666718
BD Rhapsody™ P1200 µL Pipette – HTX[c]	BD Biosciences	666719
Temperature-controlled centrifuge	Major supplier	–
Eppendorf ThermoMixer® C	Eppendorf	5382000023
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Microcentrifuge for 0.2-mL tubes	Major supplier	–
Qubit™ 3.0 Fluorometer	Thermo Fisher Scientific	Q33216
Agilent® 2100 Bioanalyzer	Agilent Technologies	G2940CA
Invitrogen™ DynaMag™-2 magnet	Thermo Fisher Scientific	12321D
Low-profile magnetic separation stand for 0.2-mL, 8-strip tubes	V&P Scientific, Inc.	VP772F4-1
Ice bucket	Major supplier	–
Vortexer	Major supplier	–
Digital timer	Major supplier	–

[a] Included with the BD Rhapsody™ Scanner.

[b] Part of the BD Rhapsody™ Xpress Package. Items can be ordered separately.

[c] Only required if not using the BD Rhapsody™ P8xP1200 µL Pipette – HTX.

Before you begin

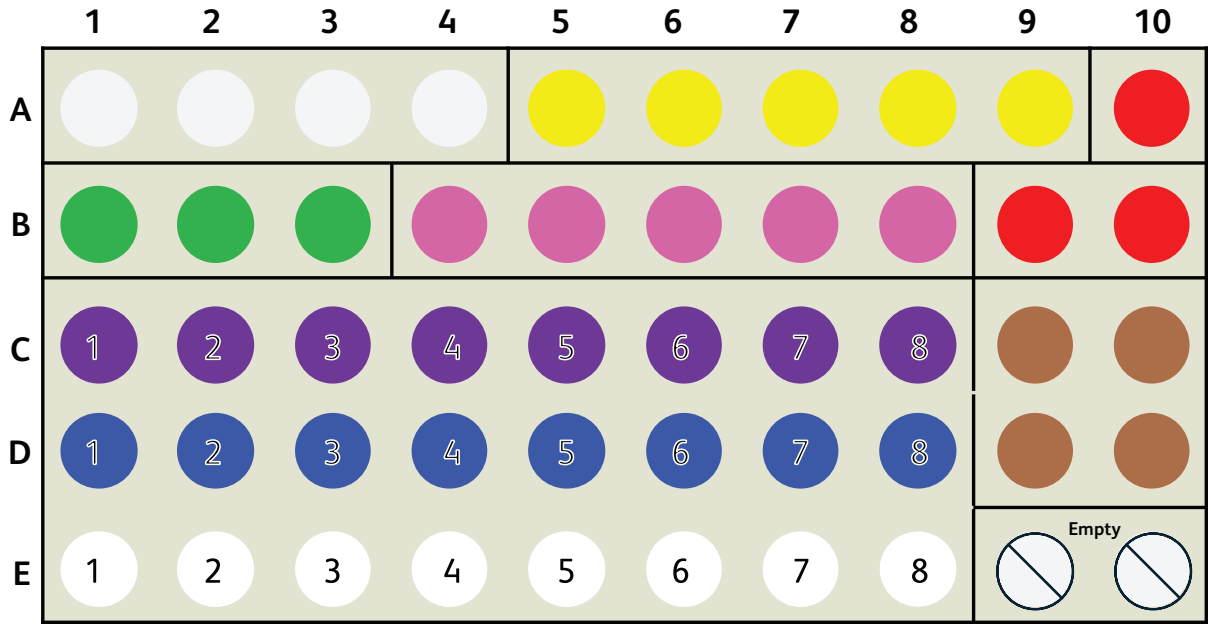
- Ensure that you have the correct kits for this protocol. Matching cap colors indicate that you have the correct kit.

BD Rhapsody™ Enhanced Cartridge Reagent Kit V3

Cap Color	Name	Quantity
	BD Rhapsody™ HT Enhanced Cell Capture Beads V3	4
	Sample buffer	1
	Cartridge wash buffer 1	1
	Cartridge wash buffer 2	1
	Lysis buffer	4
	Bead wash buffer	1
	Waste collection container	4
	1M DTT	1

BD Rhapsody™ cDNA Kit

Cap Color	Name	Quantity
	RT buffer	1
	RT 0.1M DTT	1
	Reverse transcriptase	1
	dNTP	1
	RNase Inhibitor	1
	Bead RT/PCR enhancer	1
	10X Exonuclease I buffer	1
	Exonuclease I	1
	Nuclease-free water	2
	Bead resuspension buffer	1



BD OMICS-One™ WTA Next Amplification Kit

Cap Color	Name	Part Number	Vial Placement
○	BD OMICS-One™ Nuclease-Free Water	51-9025552	A1–A4
●	BD OMICS-One™ WTA Extension Buffer	51-9025488	A5
●	BD OMICS-One™ WTA Extension Primer	51-9025467	A6
●	BD OMICS-One™ dNTP Mixture	51-9025491	A7
●	BD OMICS-One™ Bead RT/PCR Enhancer	51-9025495	A8
●	BD OMICS-One™ WTA Extension Enzyme	51-9025499	A9
●	BD OMICS-One™ AbSeq Primer	51-9025468	A10
●	BD OMICS-One™ PCR Master Mix	51-9025466	B1
●	BD OMICS-One™ Universal Oligo	51-9025553	B2
●	BD OMICS-One™ WTA Amplification Primer	51-9025469	B3
●	BD OMICS-One™ Elution Buffer	51-9025554	B4–B8
●	BD OMICS-One™ Sample Tag PCR1 Primer	51-9025470	B9
●	BD OMICS-One™ Sample Tag PCR2 Primer	51-9025471	B10
●	BD OMICS-One™ Bead Resuspension Buffer	51-9025555	C9, C10, D9, D10
●	BD OMICS-One™ Library Forward Primer 1–8	See Part numbers for primers in rows C–E (page 15)	C1–C8
●	BD OMICS-One™ WTA Library Reverse Primer 1–8		D1–D8
○	BD OMICS-One™ Multiomic Library Reverse Primer 1–8		E1–E8

Part numbers for primers in rows C–E

Name	Part Number
BD OMICS-One™ Library Forward Primer 1	51-9025472
BD OMICS-One™ Library Forward Primer 2	51-9025473
BD OMICS-One™ Library Forward Primer 3	51-9025474
BD OMICS-One™ Library Forward Primer 4	51-9025475
BD OMICS-One™ Library Forward Primer 5	51-9025476
BD OMICS-One™ Library Forward Primer 6	51-9025477
BD OMICS-One™ Library Forward Primer 7	51-9025478
BD OMICS-One™ Library Forward Primer 8	51-9025479
BD OMICS-One™ WTA Library Reverse Primer 1	51-9025480
BD OMICS-One™ WTA Library Reverse Primer 2	51-9025600
BD OMICS-One™ WTA Library Reverse Primer 3	51-9025482
BD OMICS-One™ WTA Library Reverse Primer 4	51-9025483
BD OMICS-One™ WTA Library Reverse Primer 5	51-9025484
BD OMICS-One™ WTA Library Reverse Primer 6	51-9025485
BD OMICS-One™ WTA Library Reverse Primer 7	51-9025486
BD OMICS-One™ WTA Library Reverse Primer 8	51-9025487
BD OMICS-One™ Multiomic Library Reverse Primer 1	51-9025489
BD OMICS-One™ Multiomic Library Reverse Primer 2	51-9025490
BD OMICS-One™ Multiomic Library Reverse Primer 3	51-9025492
BD OMICS-One™ Multiomic Library Reverse Primer 4	51-9025493
BD OMICS-One™ Multiomic Library Reverse Primer 5	51-9025494
BD OMICS-One™ Multiomic Library Reverse Primer 6	51-9025496
BD OMICS-One™ Multiomic Library Reverse Primer 7	51-9025497
BD OMICS-One™ Multiomic Library Reverse Primer 8	51-9025498

Multiomic ATAC-Seq Amplification Kit

Cap Color	Name	Quantity
🟢	Ligation buffer	1
🟢	Ligase	1
⚪	Nuclease-free water	1
⚪	ATAC-Seq library forward primer	1
🔴	ATAC-Seq library reverse primer 1	1
🔴	ATAC-Seq library reverse primer 2	1
🔴	ATAC-Seq library reverse primer 3	1
🔴	ATAC-Seq library reverse primer 4	1
🔴	ATAC-Seq library reverse primer 5	1
🔴	ATAC-Seq library reverse primer 6	1
🔴	ATAC-Seq library reverse primer 7	1
🔴	ATAC-Seq library reverse primer 8	1
🟢	RNase inhibitor	1
⚪	0.1M DTT, molecular biology grade	1
⚪	Bead resuspension buffer	1
🟣	Elution buffer	1
🔵	Splint oligo removal buffer	2
⚪	PCR master mix	1

ATAC-Seq Tagmentation and Supplemental Reagents Kit

Cap Color	Name	Quantity
●	Tagmentase	1
●	Tagmentation buffer	1
●	10X PBS	1
●	Digitonin 2%	1
●	Tween 20 10%	1
○	Nuclease-free water	1
●	Universal ATAC-Seq splint oligo	1
●	Splint oligo annealing buffer	1
○	Splint-bead wash buffer	1
●	Nuclei buffer	2
○	Proteinase K, molecular biology grade	1

- Thaw reagents (not enzymes) in the BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit (Cat. No. 571201) and BD Rhapsody™ Multiomic ATAC-Seq Amplification Kit (Cat. No. 571361) at room temperature
(15–25 °C), and then place on ice. Keep enzymes at –25 °C to –15 °C.

Only thaw the reagents needed for the day.

- Dilute 2% Digitonin to 1% Digitonin with nuclease-free water.
- Prepare tagmentation buffer with dimethylformamide (DMF): Thaw and transfer 200 µL of tagmentation buffer into a new 1.5-mL LoBind® tube, add 50 µL of 100% DMF into the tube, and mix by vortexing. Tagmentation buffer with DMF can be stored at –25 °C to –15 °C for later use.
- Place on ice the following components of the BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052):
  - Sample buffer
  - 1M DTT
  - Bead wash buffer
  - BD Rhapsody™ Enhanced Cell Capture Beads V3
- Visually inspect the lysis buffer for any precipitation. If precipitation is not present, leave the lysis buffer at room temperature (15–25 °C) until ready to use. 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, continue leaving the lysis buffer at room temperature until ready to use.

- Open the tube while holding the DTT tube vertically. The solution is overlain with an inert/non-oxygen-containing gas. A non-vertical tube will allow the inert gas to pour off. After opening the DTT tube once, seal and store the tube at –25 °C to –15 °C.
- Thaw DyeCycle™ Green at room temperature (15–25 °C). Follow the manufacturer's instructions and protect it from light.
- For a single cartridge workflow, adhere to the specified buffer volume as recommended in the *BD Rhapsody™ Protocol for Single Cell Capture and cDNA Synthesis*.
- When conducting the experiment in accordance with the *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-Free Workflow*, it is essential to utilize a thermomixer for the **Bead Agitation** step. It is important to keep the cartridge leveled.

Best practices

- Use low-retention filtered pipette tips.
- Use wide-bore tips when handling nuclei.
- It is important to keep the nuclei isolation on ice at all times and use RNase Inhibitor to keep RNA intact.
- When working with BD Rhapsody™ Enhanced Cell Capture Beads, use low-retention filtered tips and LoBind® tubes. Never vortex the beads. Pipet-mix only.
- Bring AMPure® XP magnetic beads to room temperature before use.
- Remove supernatants without disturbing AMPure® XP magnetic beads.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells and nuclei.
- For a complete list of materials for the BD Rhapsody™ system, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID 23-24987).

The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052) must be used for this protocol. The BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit (Cat. No. 571201) is not compatible with the BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. No. 664887).

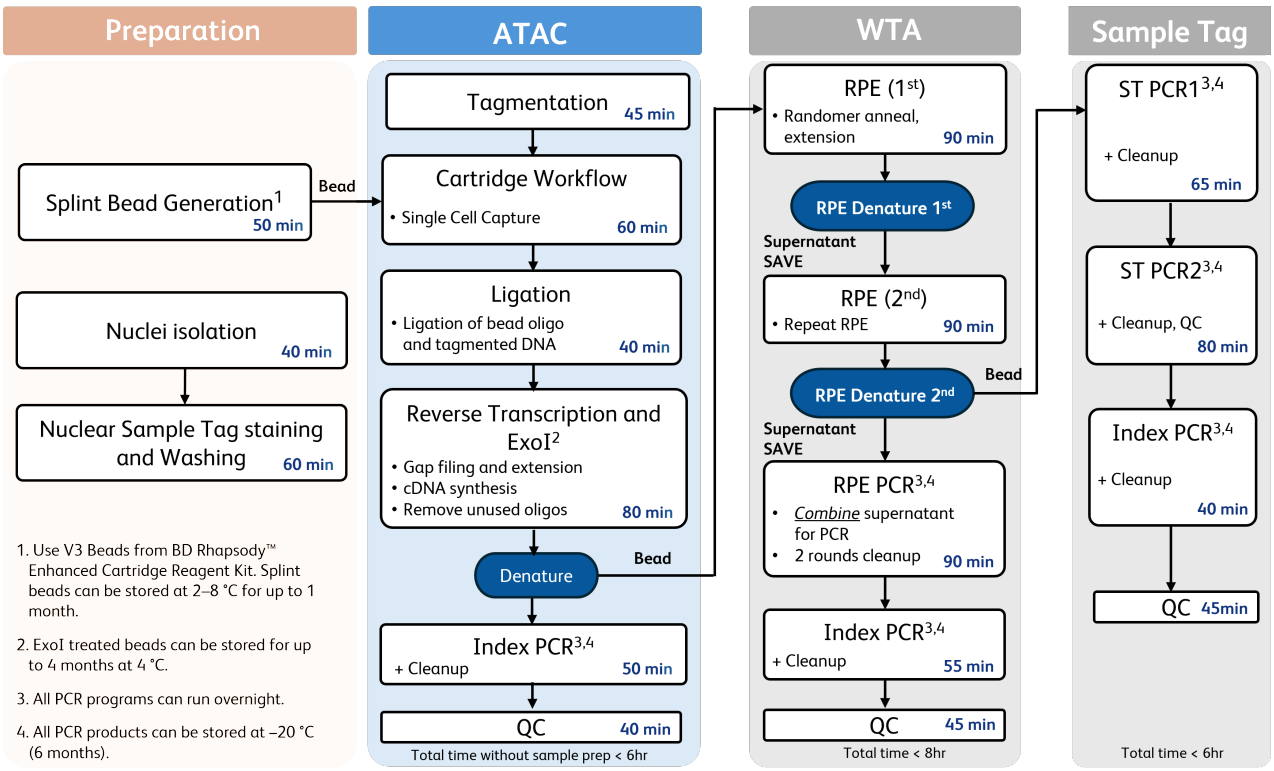
Additional documentation

- *BD Rhapsody™ Preparing Single-Cell Suspensions Protocol* (Doc ID: 23-24126)
- *BD Rhapsody™ HT Xpress System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID: 23-24983)
- *BD Rhapsody™ BD OMICS-One™ WTA Next Library Preparation Protocol* (Doc ID: 23-24991)
- *BD Rhapsody™ Sequence Analysis Pipeline User's Guide* (Doc ID: 23-24580)

Safety information

For safety information, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID: 23-24987).

Time considerations



Procedure

The range of intended total nuclei load on a cartridge is between 1,000 to 50,000 nuclei for this protocol. Nuclei load below or above this recommended range may not be suitable for current protocol configuration. Follow the procedures listed in this section.

The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052) must be used for this protocol.

1. Single-cell capture and ATAC library generation

1.1 Splint bead generation

Summary:

- Prepare splint beads
- Store on ice if proceeding on the same day or at 4 °C for up to 1 month

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟤	BD Rhapsody HT Enhanced Cell Capture Beads V3	91-1294	Keep on ice.	4 °C
⚪	Splint-bead wash buffer	51-9023092	Equilibrate to room temperature 30 minutes before use.	–20 °C
🔵	Splint oligo annealing buffer	51-9023090		
🔵	Universal ATAC-Seq splint oligo	51-9023087		
Place on ice:				
⚪	Sample buffer	650000062	Keep on ice.	4 °C
Obtain:				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 25 °C and 70 °C				

Procedure steps:

1. Set the thermomixers to 25 °C and 70 °C.
2. Obtain BD Rhapsody™ Enhanced Cell Capture Beads V3.
3. Place the tube on a magnet rack to magnetize beads down until the solution is clear.
4. Remove and discard the supernatant.
5. Remove the tube from the magnet rack and pipet 1 mL of splint-bead wash buffer to the tube.
6. Resuspend beads by slowly pipetting up and down 10 times.
7. Transfer the resuspended beads into a new 1.5-mL LoBind® tube.
8. Place the tube on a magnet rack to magnetize beads down until the solution is clear.
9. Remove and discard the supernatant.
10. Remove the tube from the magnet rack and add the following into the tube.

Color	ATAC-Seq Tagmentation and Supplemental Kit Component	Volume (µL)
○	Splint-bead wash buffer	160
●	Splint oligo annealing buffer	20
●	Universal ATAC-Seq splint oligo	20

11. Briefly centrifuge, then resuspend the beads by slowly pipetting up and down 10 times.
12. Place the tube in the thermomixer at **70 °C** and incubate for **5 minutes** with 1200 rpm mixing.
13. Transfer the tube to the thermomixer at **25 °C** and incubate for **30 minutes** with 1200 rpm mixing.
14. Remove the tube from the thermomixer, briefly centrifuge, and place the tube on a magnet rack to magnetize the beads down until the solution is clear.
15. Remove and discard the supernatant.
16. Remove the tube from the magnet rack and add **1 mL** of splint-bead wash buffer.
17. Resuspend the beads by slowly pipetting up and down 10 times.
18. Place the tube on the magnet rack to magnetize the beads down until the solution is clear.
19. Remove and discard the supernatant.
20. Repeat wash with 1 mL of splint-bead wash buffer 2 more times for a total of **three washes**.
21. After the final wash, resuspend the beads in **380 µL** of cold sample buffer by slowly pipetting up and down 10 times.
22. Store on ice if proceeding on the same day, or at 2–8 °C for up to **1 month**.

1.2 Nuclei preparation

Summary:

- Please refer to *BD Rhapsody™ System Nuclei isolation protocol* (Doc ID: 23-24852)
- Prepare nuclei suspension for tagmentation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
DyeCycle™ Green				–20 °C
Place on ice:				
●	Nuclei buffer	51-9023091	After thawing, keep on ice.	–20 °C
●	RNase inhibitor	51-9024039	Keep on ice.	
○	0.1M DTT, molecular biology grade	51-9022688	After thawing, keep on ice.	
○	BD OMICS-One™ nuclei buffer	572808		
○	Sample buffer	650000062	Keep on ice.	4 °C
Obtain:				
Ice bucket				
1.5-mL DNA LoBind® tubes				
Wide-bore tips				
5-mL polystyrene Falcon® tube				
Set up:				
BD Rhapsody™ scanner				

Procedure steps:

Reagents required for nuclei isolation are not included in the kits.

Labeling nuclei with Sample Tags

1. Prepare ATAC-SMK wash buffer by combining the following reagents in a new 5.0-mL LoBind® tube . Each sample needs 3 mL of ATAC-SMK buffer for Sample Tag staining and washing.

   ATAC-SMK wash buffer

Component	Volume (µL)
BD OMICS-One™ nuclei buffer	2970
10% Tween 20*	30
Total	**3000**

   *10% Tween 20 can be made by dilution with nuclease-free water.

2. Prepare modified nuclei buffer by combining the following reagents in a new 1.5-mL LoBind® tube. Pipet-mix for 10 times and keep it on ice.

Modified nuclei buffer	For 1 Sample (µL)	For 2 Sample (µL)	For 4 Sample (µL)	For 6 Sample (µL)
BD OMICS-One™ nuclei buffer	241.25	482.5	868.5	1254.5
BD RNase inhibitor	6.25	12.5	22.5	32.5
0.1 M DTT	2.5	5.0	9.0	13.0
Total	**250**	**500**	**900**	**1300**

3. In a 5-mL polystyrene Falcon® tube, resuspend **200,000 to 1 million** nuclei pellet in **200 µL** modified nuclei buffer.
4. Briefly centrifuge the nuclear Sample Tag tubes to collect the contents at the bottom.
5. For each sample, add **2 µL** of nuclear Sample Tag reagent to the nuclei suspension tube. Gently pipet-mix with a wide bore tip for 10 times.
6. Incubate the samples **on ice** for **30 minutes**.

Washing labeled nuclei

Sufficient post-labeling washing is important for reducing noise that comes from residual unbound antibodies being captured onto 3' capture beads during single-nuclei capture. However, some nuclei loss occurs with each additional wash. Users can choose to perform more washes depending on the abundance of their sample.

1. Pipet **2 mL** of ATAC-SMK wash buffer to labeled nuclei and pipet-mix for 10 times.
2. Centrifuge each tube at **500*g*** for **5 minutes** at **4 ˚C** to pellet the nuclei.
3. Uncap each tube and invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
4. (Optional) Repeat steps 1–3 once more for a total of 2 washes.
5. Resuspend the Sample Tag labeled nuclei pellet in modified nuclei buffer targeting for 10,000 nuclei/µL. Gently pipet-mix with a wide bore tip for 10 times and keep on ice.

Adjust resuspension volume to target 10,000 nuclei/µL. E.g. for 500K nuclei at 50% recovery, resuspend with 25 µL modified nuclei buffer. Nuclei recovery can vary based on your sample types.

Prepare nuclei suspension for tagmentation

1. Dilute an aliquot of sample-tagged nuclei for nuclei counting.
   a. Depending on the number of samples, make the master mix for sample buffer with dye.

      Do not stain entire nuclei with DyeCycle™ Green.

   b. In a new 1.5-mL tube, prepare the nuclei counting buffer by combining the following components:

Component	Volume (µL)
Cold sample buffer	190
5 mM DyeCycle™ Green*	1.0
Total	**191**

*0.3 mM DRAQ7™ can be used as substitute for 5 mM DyeCycle™ Green

   c. For each sample, add 18 µL master mix of sample buffer with dye into a new 1.5mL LoBind® tube.
   d. Ensure the nuclei are well suspended by gently pipet-mixing using wide-bore tips.
   e. Pipet **2 µL** of the nuclei suspension into the tube with **18 µL** master mix. Keep the remaining nuclei on ice.
   f. Gently pipet-mix with a wide-bore tip 5 times and incubate **on ice** for **5 minutes** to stain the nuclei, protected from light.
2. Count the stained nuclei immediately using the BD Rhapsody™ Scanner.
   a. Ensure the stained nuclei are well suspended by gently pipet-mixing.
   b. Pipet 10 µL into INCYTO disposable hemocytometer and count using the scanner.

c. Multiply the reading by 10 to calculate the concentration of unstained nuclei.

3. Pool Sample Tag labeled nuclei at a desired ratio. Multiplexed nuclei are ready for tagmentation.
   - If unstained nuclei concentration is >10,000 nuclei/µL, dilute the nuclei to 10,000 nuclei/µL with modified nuclei buffer and keep on ice.
   - If the unstained nuclei concentration is <10,000 nuclei/µL, keep on ice. Adjust the nuclei volume and nuclease-free water in the tagmentation reaction.

1.3 Tagmentation

Summary:

- Prepare tagmentation mix without Tagmentase and nuclei
- Pipet-mix and add Tn5 tagmentase
- Resuspend the nuclei
- Add the nuclei and start the reaction
- Stop the reaction and count the nuclei

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Tagmentation buffer	51-9023088	Equilibrate to room temperature 30 minutes before use. Add DMF to tagmentation buffer. Centrifuge briefly. Keep on ice until ready.	–20 °C
●	10X PBS	51-9023089		
●	Digitonin 2%	51-9023085		
●	Tween 20, 10%	51-9023084		
○	Nuclease-free water	51-9023086		
DyeCycle™ Green				–20 °C
Place on ice:				
Isolated nuclei				
○	Sample buffer	650000062	Keep on ice.	4 °C
●	RNase inhibitor	51-9024039	Keep on ice.	–20 °C
Leave in freezer until ready to use:				
●	Tagmentase	51-9023079	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Falcon® 5-mL round bottom polystyrene test tube with cell strainer snap cap				
Ice bucket				
DMSO				
1.5-mL DNA LoBind® tubes				
Wide-bore tips				
Set up:				
BD Rhapsody™ scanner				

Item	BD Part Number	Preparation and Handling	Storage
Thermomixer at 37 °C (no shaking)			

Procedure steps:

1. Set a thermomixer to 37 °C.
2. Prepare tagmentation buffer with dimethylformamide (DMF) if it was not already added:
   a. If DMF is already added, thaw at room temperature.
   b. Thaw and transfer **200 µL** of tagmentation buffer into a new 1.5-mL LoBind® tube.
   c. Add **50 µL** of 100% DMF into the tube, and mix by vortexing.

   Tagmentation buffer with DMF can be stored at –25 °C to –15 °C for later use.

3. In a new 1.5-mL LoBind® tube, add the following reagents in order.
   a. Pipet-mix the buffer and Tn5 tagmentase 10 times before adding nuclei.
   b. Ensure the nuclei are well suspended by gently pipet-mixing with a wide bore tip. Add 50,000 nuclei.
   c. Gently pipet-mix 5–10 times with a wide-bore tip.

Tagmentation mix

Cap	Kit Component	Volume for 50,000 nuclei (µL)
●	Tagmentation buffer with DMF	25
○	Nuclease-free water	11.75[a]
●	10X PBS	2
●	RNase inhibitor	1.25
●	Digitonin 1%[b]	0.5
●	Tween 20, 10%	0.5
●	Tagmentase	4
	Nuclei	5[a]
Total volume		50

[a] If the nuclei concentration is less than 10,000 nuclei/µL, adjust the volume of nuclei and nuclease-free water.

[b] 1% Digitonin is diluted from 2% stock with nuclease-free water.

Successful tagmentation has been performed with 50,000 nuclei in 50 µL reaction. For scaling up and down, see Tagmentation reaction scaling up and down table in the appendix.

4. Incubate the reaction at **37 °C** for **30 minutes** in a thermomixer **without shaking**.

During the incubation time, start priming the cartridge by following step 1 in section BD Rhapsody™ cartridge workflow (no scan other than indicated) (page 31).

5. Prepare modified cold sample buffer with RNase inhibitor as in the following table. Pipet-mix 10 times and keep on ice. If multiple samples are processed, scale it up (1 mL/sample).

Modified sample buffer with RNase inhibitor

Color	Kit Component	Volume
○	Sample buffer	1 mL
●	RNase inhibitor	25 µL

6. After incubation, add modified cold sample buffer into the Tagmentation mix.
   a. If tagmented nuclei number is different from desired single cell loading number, add **325 µL** of modified cold sample buffer into the tagmentation mix.
   b. If proceeding entire tagmented nuclei to single cell capture without counting, add **310 µL** of modified cold sample buffer into the Tagmentation mix.
7. Gently pipet-mix 5 times and keep on ice.
8. Take the cell strainer top off the 5-mL round bottom tube and wet it with 50 µL of sample buffer.
9. Put the top back onto the tube and filter the tagmented nuclei through a Falcon tube with cell strainer cap. Place the tube on ice.
10. **Optional:** stain an aliquot of tagmented nuclei and count the stained nuclei immediately using the BD Rhapsody™ Scanner.

DO NOT STAIN THE ENTIRE SAMPLE.

Nuclei staining dye can impact ATAC-seq data. Consequently, no cartridge scanner metrics will be collected.

   a. Ensure the nuclei are well suspended by gently pipet-mixing.
   b. Pipet **15 µL** of the nuclei suspension into a new 1.5-mL LoBind® tube. Keep the remaining nuclei suspension on ice.
   c. Pipet **0.4 µL** of 5-times diluted DyeCycle™ Green* (1 mM) into the tube containing the 15 µL nuclei aliquot.

*Dilute 5 mM DyeCycle™ Green 5 fold to 1 mM with DMSO. 0.06 mM DRAQ7™ can be used as substitute.

   d. Pipet-mix 5 times with wide-bore tip and incubate **on ice** for **5 minutes** to stain the nuclei, protected from light.
   e. Count the stained nuclei immediately using the BD Rhapsody™ Scanner.
   f. Ensure the stained nuclei are well suspended by gently pipet-mixing.
   g. Pipet 10 µL into INCYTO disposable hemocytometer and count using the scanner.
   h. Viability information is not applicable. Use the concentration to calculate loading dilution and dilute with modified sample buffer.

1.4 BD Rhapsody™ cartridge workflow (no scan other than indicated)

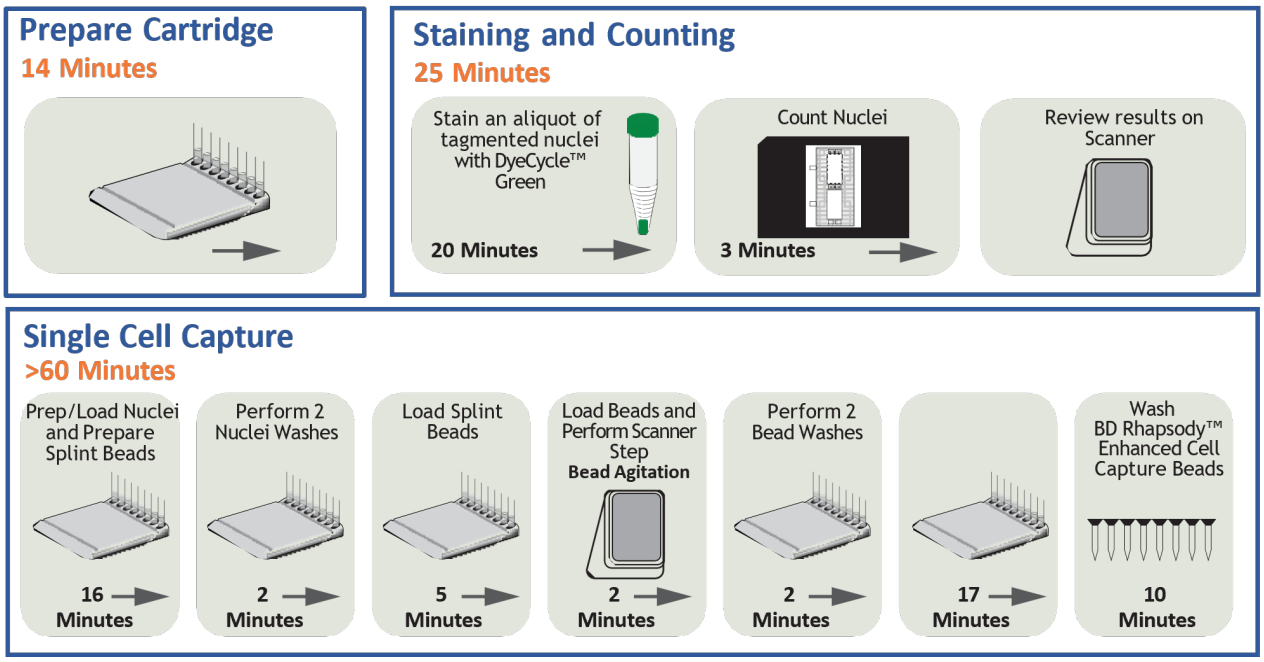
Summary:

- Prepare cartridge
- Staining and counting
- Single cell capture

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
○	Cartridge wash buffer 1	650000060	Equilibrate to room temperature 30 minutes before use.	4 °C
○	Cartridge wash buffer 2	650000061		
○	Lysis buffer	650000064		
Place on ice:				
Nuclei suspension and splint beads				
●	RNase inhibitor	51-9024039	Keep on ice.	–20 °C
○	Sample buffer	650000062		4 °C
○	1M DTT	650000063		
○	Bead wash buffer	650000065		
Leave in freezer until ready to use:				
○	Proteinase K, Molecular Biology Grade	51-9022689	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
BD Rhapsody™ 8-Lane Cartridge				
BD Rhapsody™ P8xP1200 µL pipette-HTX				
Ice bucket				
1.5 mL DNA LoBind® tubes or 96-deep-well plate				
Cluster tube 8-tube strip				
1.5 mL PCR tube magnetic rack				
Set up:				
BD Rhapsody™ scanner				
Incubator at 37 °C				

Procedure steps:



1. Prime the cartridge

   a. Prior to priming the cartridge, scan at least one lane of the empty cartridge for Cell Load scan. For detailed instructions, refer to *BD Rhapsody™ HT Single-Cell Capture and Analysis System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID: 23-24984).

   b. Aliquot 100% ethyl alcohol and cartridge reagent (kept at room temperature) buffers in 10-mL or 25-mL reagent reservoirs based on the number of lanes used, as instructed by the following table. Do not aliquot for single lane.

Component	For 1 lane (mL)	For 2 lane (mL)	For 3 lane (mL)	For 4 lane (mL)	For 5 lane (mL)	For 6 lane (mL)	For 7 lane (mL)	For 8 lane (mL)
1	0.05	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2	0.76	3.50	5.25	7.00	8.75	10.50	12.25	14.00
3	0.38	2.00	3.00	4.00	5.00	6.00	7.00	8.00

   c. Place waste collection container and cluster tube in the BD Rhapsody™ HT Xpress System.

   d. Carefully peel off the seal on the cartridge inlet of the lanes to be used.

   e. Prime the cartridge using the following table steps with BD Rhapsody™ P8xP1200 µL pipette:

Step number	Material to load	Volume (µL/lane)	Pipette mode	Incubation at room temperature
1	100% ethyl alcohol	50	EtOH	N/A

Step number	Material to load	Volume (µL/lane)	Pipette mode	Incubation at room temperature
2	Air	380	Prime/Wash	N/A
3	Room temp. Cartridge wash buffer 1	380	Prime/Wash	1 minute
4	Air	380	Prime/Wash	N/A
5	Room temp. Cartridge wash buffer 1	380	Prime/Wash	3 minutes
6	Air	380	Prime/Wash	N/A
7	Room temp. Cartridge wash buffer 2	380	Prime/Wash	≤4 hours

2. Prepare single-nuclei suspension for cartridge loading:

Nuclei loading efficiency can be different based on your sample types. Observed nuclei recovery rate for cell line sample is ~60–70% and for PBMC sample is ~40–50%. Please load targeted nuclei # based on your sample nuclei recovery rate estimate.

- If the entire tagmented nuclei will be loaded, transfer each tube of prepared nuclei suspension into a 96-deep-well plate for multiple lane loading. Keep the 96-deep-well plate on ice.
- **Optional:** Use the BD Rhapsody™ Scanner to calculate the number of nuclei for cartridge loading. Select Sample Calculator.
  a. Select the correct cartridge type. For the BD Rhapsody™ 8-Lane Cartridge, use 0120.
  b. Calculate the volumes of tagmented nuclei and modified sample buffer with RNase Inhibitor needed to prepare a nuclei suspension of 380 µL (this volume is for one lane).
  c. Prepare 380 µL nuclei suspension for cartridge loading by mixing unstained tagmented nuclei with modified cold sample buffer with RNase Inhibitor according to the displayed volumes on the scanner. Ensure the stock solution of each sample is well suspended by gently pipet-mixing with a wide-bore tip before pooling. Keep the nuclei suspension on ice.

3. Load tagmented nuclei in the cartridge:
   a. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P8xP1200 µL pipette:

Material to load	Volume (µL/lane)	Pipette mode
Air	380	Prime/Wash
• Gently pipet mix with a multi-channel pipette to completely resuspend the nuclei. • Set the BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) to Load mode. • Immediately load.		
Nuclei suspension	320	Load

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

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

c. Optional: Image the nuclei in the cartridge in case for the manual analysis (See Appendix (page 93) for calculation). Automatic analysis is not available. Perform the scanner step: Cell Load. For more information, see the instrumentation user guide.

4. Wash the loaded nuclei with cold sample buffer:

Do not omit this step. It is necessary to obtain good ATAC-Seq data.

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

b. Set the BD Rhapsody™ P8x1200 µL pipette to Prime/Wash mode.

c. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P8x1200 µL pipette:

Material to load	Volume (µL/lane)	Pipette mode
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash

5. Load and wash cell-capture beads:

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

b. Set the BD Rhapsody™ P8xP1200 µL pipette to **Prime/Wash** mode.

c. Bring the splint beads generated from the Splint bead generation steps.

d. Load the cartridge with materials listed below using the BD Rhapsody™ P8xP1200 µL 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 beads. • Set the BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) to Load mode. • With a new set of pipette tips, immediately load the beads. Check the pipette tips to make sure that there are no air bubbles inside the tips before loading. Otherwise, dispense the beads into the 96-deep well plate and aspirate with a new set of pipette tips.		
Splint beads	320	Load

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

f. Perform scanner step: **Bead Agitation**.

g. After bead agitation is complete, tap **OK**, then **Eject**. Remove the cartridge from the scanner.

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

i. Set the BD Rhapsody™ P8xP1200 µL pipette to Prime/Wash mode.

j. Load the cartridge with materials listed below using the BD Rhapsody™ P8xP1200 µL 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

6. Lyse nuclei:

Lysis buffer should be kept at room temperature until ready to use!

a. Add **75.0 µL** of 1 M DTT to one room-temperature 15-mL lysis buffer bottle and briefly vortex mix. Use the lysis buffer with DTT within 24 hours, and then discard.

b. Pipette **0.5 mL** of lysis buffer with DTT into a new 1.5-mL LoBind® tube. Add **25 µL** of Proteinase K to the tube immediately before the lysis step, and gently pipet-mix 5 times.

0.5 mL is enough for one lane. Scale up proportionally if multiple lanes are used.

c. Set the BD Rhapsody™ P8xP1200 µL pipette to Lysis mode.

d. Load the cartridge with materials listed using the BD Rhapsody™ P8xP1200 µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Lysis buffer with DTT and Proteinase K	280	Lysis

e. Carefully remove the cartridge from the BD Rhapsody™ HT Xpress System. Slowly transfer the cartridge into an incubator at **37 ˚C** and incubate for **10 minutes**. Maintain the recommended lysis time for best performance.

It is important to keep the cartridge leveled.

7. Retrieve cell-capture beads:
   a. Ensure the cluster tube 8-tube strip is placed into the BD Rhapsody™ HT Xpress System drawer. Label the tubes appropriately.
   b. Ensure that the BD Rhapsody™ P8xP1200 µL pipette is set to **Retrieval** mode.
   c. Move the front slider to BEADS on the BD Rhapsody™ HT Xpress System.
   d. Carefully bring the cartridge from the 37 °C incubator to the BD Rhapsody™ HT Xpress System and allow the cartridge to cool down for **5 minutes**.
   e. Gently pull the top RETRIEVAL slider toward and on top of the cartridge.
   f. Leave the retrieval magnet in the down position for **1 minute**.
   g. Aspirate **1,000 µL** lysis buffer with DTT using the BD Rhapsody™ P8xP1200 µL pipette.
   h. Press down on the BD Rhapsody™ P8xP1200 µL pipette to seal against the gasket.
   i. Push back the top RETRIEVAL magnet, and immediately load 1,000 µL lysis buffer with DTT.
   j. Remove the pipette from the gasket and purge the tips.
   k. Move the front slider to OPEN and remove the cluster tube with the bottom adapter to a flat, secure surface.
8. Transfer the cell-capture beads to a new tube for incubation and incubate in lysis buffer:
   a. Remove the cluster tube from the bottom adapter.
   b. Gently pipet-mix the beads and transfer into a new 1.5-mL LoBind® tube. Keep on ice.
   c. If beads are still left in the cluster tube, add 100 µL of lysis buffer with DTT, rinse the cluster tube, and transfer into the 1.5-mL LoBind® tube from the previous substep.
   d. Place the tube on magnet for 2 minutes and remove all supernatant.
   e. Resuspend the beads in **1.0 mL** lysis buffer with DTT.
   f. Incubate the tube in thermomixer at **37 °C**, 1200 rpm for **15 minutes**.
9. Wash cell-capture beads:
   a. Place the tube on a magnet rack for 2 minutes.
   b. Remove and discard the supernatant. Avoid leaving lysis buffer or bubbles in the tube. Otherwise, the lysis buffer might cause the ligation reaction to fail.
   c. Remove the tube from the magnet, and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix.
   d. Place the tube on the magnet rack for 2 minutes. Remove and discard the supernatant.
   e. Remove the tube from magnet, and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix, and place on ice.

STOP **Optional:** Store cell-capture beads in bead wash buffer at 4 °C overnight and continue to ligation the next day.

This condition has only been validated for select sample types. Use only if extended storage is required.

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 shown in the following table, depending on the number of lanes used. Do not aliquot for single lane.

Component	1 lane (mL)	2 lanes (mL)	3 lanes (mL)	4 lanes (mL)	5 lanes (mL)	6 lanes (mL)	7 lanes (mL)	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™ P8xP1200 µL Pipette (or BD Rhapsody™ P1200 µL 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 unused lanes.
5. Make sure the seals of the unused lanes 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.

Partially used cartridges can be stored for up to 1 year at room temperature.

1.5 Ligation

Summary:

- Prepare Ligation mix
- Perform Ligation reaction

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Ligation buffer	51-9023094	After thawing, keep on ice.	–20 °C
○	Nuclease-free water	51-9023086		
Place on ice:				
●	RNase inhibitor	51-9024039	Keep on ice.	–20 °C
Leave in freezer until ready to use:				
●	Ligase	51-9023093	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Washed capture beads				4 °C
Ice bucket				
1.5 mL DNA LoBind® tubes				
1.5 mL PCR tube magnetic rack				
Set up:				
Thermomixer at 25 °C				

Procedure steps:

1. Set the thermomixers to 25 °C, 42 °C, and 60 °C.
2. In a new 1.5-mL or 2.0-mL LoBind® tube, add the following components:

Ligation mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
●	Ligation buffer	20	22	88	176
●	Ligase	10	11	44	88
●	RNase inhibitor	5	5.5	22	44
○	Nuclease-free water	165	181.5	726	1452
	Total	**200**	**220**	**880**	**1760**

3. Gently vortex Ligation mix, briefly centrifuge and place on ice.
4. Place the tube of washed cell-capture beads on a magnet rack for 2 minutes.
5. Remove and discard the supernatant.
6. Remove the tube from the magnet and pipet **200 µL** of Ligation mix into the tube.
7. Resuspend the beads by pipet-mixing 10 times.
8. Transfer the whole reaction into a new 1.5-mL LoBind® tube.
9. Incubate the tube in the thermomixer at **25 °C** for **30 minutes** with 1,200 rpm mixing.
10. Proceed to the Reverse Transcription (RT) (page 40) steps immediately.

1.6 Reverse Transcription (RT)

Summary:

- Prepare reverse transcription (RT) mix
- Perform RT reaction

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟠	RT buffer	650000067	Equilibrate to room temperature 30 minutes before setting up cDNA synthesis. Centrifuge briefly.	–20 °C
🟠	dNTP	650000077		
🟠	0.1 M DTT	650000068		
⚪	Nuclease-free water	650000076		
Place on ice:				
⚫	Bead RT/PCR enhancer	91-1082	Centrifuge briefly before adding to mix.	–20 °C
Leave in freezer until ready to use:				
🟠	RNase inhibitor	650000078	Centrifuge briefly before adding to mix.	–20 °C
🟠	Reverse transcriptase	700026321		
Obtain:				
Ligated beads				
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 42 °C				

Procedure steps:

1. In a new 1.5-mL or 2.0-mL LoBind® tube, add the following components. Gently vortex mix, briefly centrifuge and place on ice.

RT mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
🟠	RT buffer	40	44	176	352
🟠	dNTP	20	22	88	176
🟠	RT 0.1M DTT	10	11	44	88
⚫	Bead RT/PCR enhancer	12	13.2	52.8	105.6
🟠	RNase inhibitor	10	11	44	88
🟠	Reverse transcriptase	10	11	44	88
⚪	Nuclease-free water	98	107.8	431.2	862.4
	Total	**200**	**220**	**880**	**1760**

2. Upon completion of the Ligation (page 38) steps, remove the tube from the thermomixer and place on the magnet for 2 minutes. Remove and discard the supernatant.
3. Remove the tube from the magnet and pipet **200 µL** of RT Mix into the tube.
4. Resuspend the beads by pipet-mixing 10 times.
5. Incubate the tube in the thermomixer at **42 °C** for **30 minutes** with 1,200 rpm mixing.

1.7 Splint oligo removal

Summary:

- Remove splint oligo
- Place on ice for 5 minutes

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Splint oligo removal buffer	51-9024041	Equilibrate to room temperature 30 minutes before use.	–20 °C
Obtain:				
RT beads				
Ice bucket				
1.5 mL DNA LoBind® tubes				
1.5 mL PCR tube magnetic rack				
Set up:				
Thermomixer at 60 °C				

Procedure steps:

1. Upon completion of reverse transcription, remove the tube from the thermomixer and place on the magnet for 2 minutes. Remove and discard the supernatant.
2. Remove the tube from the magnet and pipet **200 µL** of splint oligo removal buffer into the tube.
3. Resuspend the beads by pipet-mixing 10 times.
4. Incubate the tube in the thermomixer at **60 ˚C** for **5 minutes** with 1,200 rpm mixing.
5. Remove the tube from the thermomixer and immediately place it **on ice** for **5 minutes**.

1.8 Exonuclease I treatment

Summary:

- Prepare Exonuclease I (ExoI) mix
- Perform ExoI reaction

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	10X Exonuclease I buffer	650000071	Equilibrate to room temperature 30 minutes before setting up ExoI. Centrifuge briefly.	–20 °C
●	Bead resuspension buffer	650000066		
○	Nuclease-free water	650000076		
Leave in freezer until ready to use:				
●	Exonuclease I	650000078	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Splint oligo removed beads				
0.5M EDTA				
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 37 °C				

Procedure steps:

1. Set the thermomixer to **37 °C**.
2. In a new 1.5-mL or 2.0-mL LoBind® tube, add the following components and gently vortex mix. Briefly centrifuge then place on ice.

Exonuclease I mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
	10X Exonuclease I buffer	20	22	88	176
	Exonuclease I	10	11	44	88
	Nuclease-free water	170	187	748	1496
	Total	**200**	**220**	**880**	**1760**

3. Remove the tube from ice, quick spin and place on the magnet for 2 minutes.
4. Remove and discard the supernatant.
5. Remove the tube from the magnet and pipet **200 µL** of Exonuclease I mix into the tube.
6. Resuspend the beads by pipet-mixing 10 times.
7. Incubate the tube in the thermomixer at **37 °C** for **30 minutes** with 1,200 rpm mixing.
8. Remove the tube from the thermomixer and add **4 µL** of 0.5M EDTA to the Exonuclease I-treated beads. Pipet-mix 10 times.
9. Briefly centrifuge and then place the tube on the magnet for 2 minutes.
10. Remove and discard the supernatant.
11. Remove the tube from the magnet and pipet **200 µL** of bead resuspension buffer into the tube. Resuspend the beads by pipet-mixing 10 times.

STOP Exonuclease I - treated beads can be stored at 2–8 °C for up to 4 months.

1.9 ATAC index PCR

Summary:

- Denature ATAC products
- Prepare ATAC PCR mix
- Amplify using ATAC index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Elution buffer	51-9023107	Equilibrate to room temperature 30 minutes before setting up index PCR. Centrifuge briefly. Keep on ice until ready.	–20 °C
○	ATAC-Seq library forward primer	51-9023097		
●	ATAC-Seq library reverse primer	Various		
○	Bead resuspension buffer	51-9023126		
Leave in freezer until ready to use:				
○	PCR master mix	51-9024048	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
ExoI treated beads				
Ice bucket				
0.2-mL PCR tubes				
1.5-mL DNA LoBind® tubes				
1.5-mL PCR tube magnetic rack				
Set up:				
Thermomixer at 95 °C (no shaking) or heat block at 95 °C				
Thermocycler with ATAC index PCR program				

Procedure steps:

1. Set a thermomixer to 95 °C.
2. Choose between using the entire sample or a sub-sample of the Exonuclease I - treated beads. If using the entire sample, skip to step 4. If using a subsample, proceed to step 3.
3. **(Optional)** Subsample the Exonuclease I - treated beads:
   - Determine the volume of beads to subsample for sequencing, based on the expected number of nuclei captured on beads in the final bead-resuspension volume.
   - Completely resuspend the beads by pipet-mixing, then pipet the calculated volume of bead suspension into a new 1.5-mL LoBind® tube. If needed, bring the total volume up to 200 µL with bead resuspension buffer.

   The remaining beads can be stored in bead resuspension buffer at 4 °C for up to 4 months.

4. Place the tube with Exonuclease I - treated beads on a magnet rack for 2 minutes. Remove and discard the supernatant.
5. Pipet **40 µL** of elution buffer to the beads. Pipet-mix.
6. Incubate the tube in the thermomixer at **95 °C** for **5 minutes** (no shaking).
7. Remove the tube from the thermomixer and immediately place the tube **on ice** for **1 minute**.
8. Remove the tube from ice, quick spin, and then place the tube on a magnet rack until the solution is clear.
9. Transfer the entire supernatant to a new 0.2-mL PCR tube. Keep on ice.
10. Pipet **40 µL** of elution buffer to the beads. Pipet-mix.
11. Incubate the tube in the thermomixer at **95 °C** for **5 minutes** (no shaking).
12. Remove the tube from the thermomixer and immediately place the tube **on ice** for **1 minute**.
13. Remove the tube from ice, quick spin and then place the tube on the magnet rack until the solution is clear.
14. Transfer the entire supernatant into the PCR tube with the previously collected 40 µL eluted supernatant. Total **80 µL** of ATAC products.
15. Resuspend the beads with **200 µL** bead resuspension buffer. Store the beads at 2–8 °C until ready for WTA library generation (up to 72 hours) as described in WTA library amplification (page 52).
16. In a new 1.5-mL tube, add the following components. Gently vortex mix, briefly centrifuge, then place on ice.

ATAC index PCR mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
○	PCR master mix	30	33	132	264
○	ATAC-Seq library forward primer	6	6.6	26.4	52.8
●	ATAC-Seq library reverse primer(1–8)[a]	6	6.6	NA	NA
	Total	**42**	**46.2**	**158.4**	**316.8**

a. For more than one ATAC library, use a different ATAC-Seq library reverse primer for each library.

17. Combine the ATAC index PCR mix with ATAC products as follows:

- For one sample, the ATAC index PCR mix includes an ATAC-Seq library reverse primer. Combine **42 µL** of the mix with **80 µL** of ATAC product. Pipet-mix 10 times, and then split the reaction volume (122 µL) into two 0.2-mL PCR tubes.
- If working with multiple samples, do not include ATAC-Seq library reverse primer into the ATAC index PCR mix. In separate tubes for each sample, combine 36 µL of the ATAC index PCR mix with 80 µL of ATAC product and 6 µL of the ATAC-Seq library reverse primer that is specifically assigned to the sample. Pipet-mix 10 times, and then split the reaction volume (122 µL) into two 0.2-mL PCR tubes.

18. Gently vortex mix and briefly centrifuge.
19. In post-amplification workspace. Run the following PCR program. (Volume = 60 µL)

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	12–16 cycles[a]	98 °C	10 seconds
Annealing		66 °C	30 seconds
Extension		72 °C	30 seconds
Final extension	1	72 °C	1 minute
Hold	1	10 °C	∞
a. Suggested PCR cycles might need to be optimized for different sample types and number of cells.			

STOP The PCR can run overnight.

Recommended number of PCR cycles

Number of cells in ATAC PCR	Suggested number of PCR cycles
>10,000	12
10,000–5,000	13
4,999–1,000	14
<1,000	16

1.10 ATAC index PCR Cleanup and Quality Check

Summary:

- ATAC index PCR cleanup
- Quality check using Qubit™ Fluorometer and BioAnalyzer/TapeStation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Elution buffer	51-9023107	Centrifuge briefly.	–20 °C
○	Nuclease-free water	51-9023086		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit™ dsDNA HS Assay Kit				
Agilent Bioanalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
ATAC Index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
1.5-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume of 80% ethyl alcohol depending on the number of samples – one sample requires 2 mL 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the ATAC index product.
5. Combine the **two** tubes of **60 µL** ATAC index product into a new 1.5-mL LoBind® tube.
6. Pipet-mix 10 times.
7. Pipet **144 µL** of AMPure® XP beads (**1.2x**) into the tube.
8. Pipet-mix 10 times.
9. Briefly centrifuge the tube.

   Avoid getting AMPure® beads on the lid of the tube. Residual AMPure® beads and PCR mix buffer can negatively impact downstream results.
10. Incubate at room temperature for **5 minutes**.
11. Place the tube on a magnet until the supernatant is clear (**<3 minutes**).
12. Remove and discard the supernatant.
13. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
14. Incubate for **30 seconds**.
15. Remove and discard the supernatant without disturbing the beads.
16. Repeat steps 13–15 once for a total of **two ethyl alcohol washes**.
17. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
18. Air-dry the beads at room temperature until the beads no longer look glossy (**~3 minutes**).

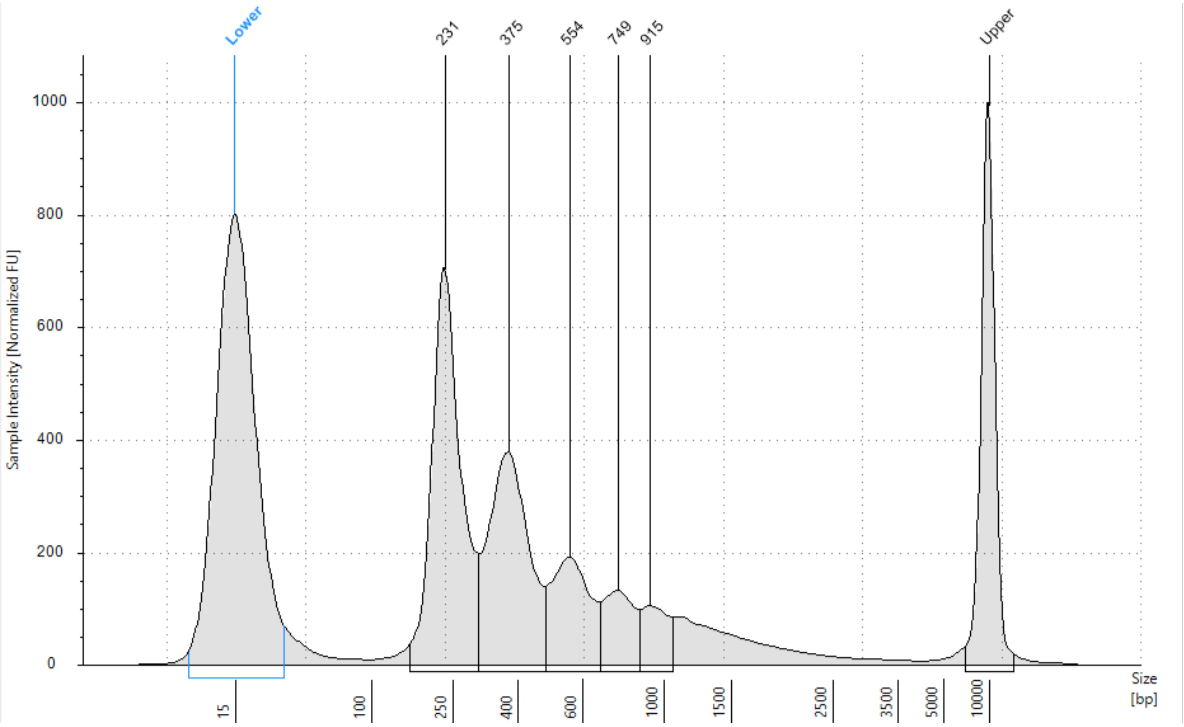
    Do not overdry the AMPure® beads after the ethanol washes. Overdried beads appear cracked.
19. Remove the tube from the magnet.
20. Pipet **40 µL** of elution buffer into the tube.
21. Pipet-mix 10 times until the beads are fully resuspended.
22. Incubate at room temperature for **2 minutes**.
23. Briefly centrifuge the tube.
24. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
25. Pipet the eluate (40 µL) into a new 1.5-mL LoBind® tube.

STOP The libraries can be stored at –20 °C for up to 3 months until sequencing.

26. Measure the concentration of each ATAC library by quantifying 2 µL of the final sequencing library with a Qubit™ Fluorometer and Qubit™ dsDNA HS assay, and perform quality control of the ATAC library using either of the following systems:
    a. Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit
    b. Agilent 4200 TapeStation system using the Agilent High Sensitivity D1000 or D5000 ScreenTape Assay
27. If needed, dilute the library to the quantitative range of the Agilent 2100 Bioanalyzer. Measure the fragment size of the library following the manufacturer's instructions.
    - If the concentration is >5 ng/µL, dilute the library to ≤5 ng/µL with elution buffer.
    - Measure the average fragment size of the ATAC libraries within the size range of 200–1,000 bp by using the Agilent Bioanalyzer with the Agilent High Sensitivity DNA Kit (Agilent Cat. No. 5067-4626). Follow the manufacturer's instructions.

    The following diagram shows a representative ATAC library trace from the Bioanalyzer and TapeStation, where the majority of the fragments are distributed between ~200–2000 bp.

Figure 1 BD Rhapsody™ ATAC Library – TapeStation high-sensitivity D5000 trace



2. WTA library amplification

Before you begin:

- Obtain beads from step 15 of ATAC index PCR (page 46).
- Thaw reagents (except for the enzymes) in the BD OMICS-One™ WTA Next Amplification Kit at room temperature (15–25 °C), then place on ice. Keep enzymes at –25 °C to –15 °C.

2.1 WTA Random Priming and Extension (RPE)

Summary:

- Prepare random priming mix and extension enzyme mix
- Anneal random primers
- Extend random primers
- Denature RPE products
- Repeat RPE (2x total)

Preparation list:

Item		BD Part Number	Preparation & Handling	Storage
Equilibrate to room temperature:				
🟡	WTA extension buffer	51-9025488	Equilibrate to room temperature 30 minutes before setting up RPE. Centrifuge briefly.	–20 °C
🟡	WTA extension primer	51-9025467		
🟡	dNTP mixture	51-9025491		
⚪	Nuclease-free water	51-9025552		
🟣	Elution buffer	51-9025554		
⚫	Bead resuspension buffer	51-9025555		
Place on ice:				
🟡	Bead RT/PCR enhancer	51-9025495	Centrifuge briefly before adding to mix.	–20 °C
Leave in freezer until ready to use:				
🟡	WTA extension enzyme	51-9025499	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
ATAC denatured beads			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Heat block at 95 °C				
Thermomixer at 37 °C (Optional)				
Thermomixer at 25 °C				
Programmed thermomixer with RPE program				

Procedure steps:

This section describes how to generate random priming products. First, random primers are hybridized to the cDNA on the BD Rhapsody™ Enhanced Cell Capture Beads, then extended with an enzyme. This hybridization and extension is repeated a second time to increase assay sensitivity.

Perform this procedure in the pre-amplification workspace.

1. Set a heat block to 95 °C, one thermomixer to 37 °C, and another thermomixer to 25 °C.

   Optional: If you are using one thermomixer, skip the 37 °C incubation step.

2. In a new 1.5-mL LoBind® tube, pipet the following reagents.

Random primer mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	WTA extension buffer	20.0	24.0	96.0	192.0
	WTA extension primer	40.0	48.0	192.0	384.0
	Nuclease-free water	114.0	136.8	547.2	1,094.4
	Total	**174.0**	**208.8**	**835.2**	**1670.4**

3. Pipet-mix the random primer mix and keep at room temperature.
4. Resuspend the beads with a pipette.
5. Place the tube with beads in a **95 °C** heat block for **5 minutes** (no shaking).
6. Briefly centrifuge the tube, then immediately place the tube in the 1.5-mL magnetic separation rack. Remove and discard the supernatant. Avoid drying out the BD Rhapsody™ Enhanced Cell Capture Beads.
7. Remove the tube from the magnet and use a low-retention tip to pipet **87 µL** of random primer mix into the tube. Pipet-mix 10 times to resuspend the beads. Save the remaining volume of random primer mix for a second RPE. Keep random primer mix at room temperature.
8. Incubate the tube in the following order:
   a. **95 °C** in a heat block (no shaking) for **5 minutes**.
   b. Thermomixer at 1,200 rpm and at **37 °C** for **5 minutes**.

      Optional: If you are using one thermomixer, skip the 37 °C incubation step.

   c. Thermomixer at 1,200 rpm and at **25 °C** for **5 minutes**.
9. Briefly centrifuge the tube and keep it at room temperature.
10. In a new 1.5-mL LoBind® tube, pipet the following reagents.

Primer extension enzyme mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
	dNTP	8.0	9.6	38.4	76.8
	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
	WTA extension enzyme	6.0	7.2	28.8	57.6
	Total	**26.0**	**31.2**	**124.8**	**249.6**

11. Pipet-mix the primer extension enzyme mix.
12. Pipet **13 µL** of the primer extension enzyme mix into the sample tube containing the beads (for a total volume of 100 µL) and keep at room temperature until ready. Save the remaining volume of primer extension enzyme mix for a second RPE. Keep primer extension enzyme mix on ice.
13. Program the thermomixer.
    a. 1,200 rpm and at **25 ˚C** for **10 minutes**.
    b. 1,200 rpm and at **37 ˚C** for **15 minutes**.
    c. 1,200 rpm and at **45 ˚C** for **10 minutes.**
    d. 1,200 rpm and at **55 ˚C** for **10 minutes**.

    Confirm "Time Mode" is set to "Time Control" before the program begins.

14. Place the sample tube containing the beads and primer extension enzyme mix in the thermomixer. Start the program set up in the preceding step.
15. Place the tube in a 1.5-mL tube magnet and remove and discard the supernatant.
16. Remove the tube from the magnet and resuspend the beads in **200 µL** of elution buffer.
17. Place the tube on a magnet until the supernatant is clear (<2 minutes).
18. Remove and discard the supernatant.
19. Remove the tube from the magnet.
20. Pipet **80 µL** of elution buffer into the tube.
21. To denature the random priming products off the beads.
    a. Pipet-mix 10 times to resuspend the beads.
    b. Incubate the tube at **95 ˚C** in a heat block for **5 minutes (no shaking)**.
    c. Slightly open the lid of the tube to release air pressure within the tube.
    d. Place the tube **on ice** for **1 minute**.
    e. Briefly centrifuge the tube.

f. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).

SAVE SUPERNATANT AT THIS STEP. Do not discard.

g. Transfer **80 µL** of the supernatant (RPE product) to a new 1.5-mL LoBind® tube.

22. Place the tube containing the RPE product on ice.
23. Repeat steps 7 to 22 to perform a second RPE.

If working with multiple samples, ensure that the supernatants are combined correctly.

24. Combine the 2 RPE products for each sample, for a total volume of **160 µL** (80 µL from 1st RPE + 80 µL from 2nd RPE).
25. Resuspend beads in bead resuspension buffer and store until ready for Sample Tag library generation.
    a. Pipet 200 µL of cold bead resuspension buffer to the tube with leftover beads.
    b. Gently resuspend the beads by pipet-mixing only. Do not vortex.
    c. Store the beads on ice or at 2–8 °C in the pre-amplification workspace until ready for Sample Tag library generation as described in Sample Tag library amplification (page 72).
26. Immediately proceed to RPE PCR.

2.2 WTA RPE PCR

Summary:

- Prepare RPE PCR mix
- Amplify using RPE PCR program

Preparation list:

Item		BD Part Number	Preparation & Handling	Storage
Equilibrate to room temperature:				
●	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up RPE PCR. Centrifuge briefly.	–20 °C
●	WTA amplification primer	51-9025469		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with RPE PCR program				

Procedure steps:

This section describes how to generate more RPE product through PCR amplification, so that there are multiple copies of each random-primed molecule.

In the pre-amplification workspace, in a new 1.5-mL LoBind® tube, pipet the following components.

RPE PCR mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
	PCR master mix	60.0	72.0	288.0	576.0
	Universal oligo	12.0	14.4	57.6	115.2
	WTA amplification primer	12.0	14.4	57.6	115.2
	Total	**84.0**	**100.8**	**403.2**	**806.4**

1. Pipet-mix the RPE PCR mix.
2. Place on ice until ready to use.
3. Add **84 µL** of the RPE PCR mix to the tube with the **160 µL** of RPE product.
4. Pipet-mix 10 times to create the RPE PCR reaction mix.
5. Split the RPE PCR reaction mix into four 0.2-mL PCR tubes with **61 µL** mix per tube.
6. Transfer any residual mix to one of the tubes.
7. Bring the tubes to the post-amplification workspace.
8. Run the following PCR program.

RPE PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	Recommended PCR cycles* 1,000–20,000: 9 cycles 20,000–30,000: 8 cycles 30,000–50,000: 7 cycles	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

*Recommended number of PCR cycles might require optimization for different sample types.

STOP The PCR can run overnight.

9. When the RPE PCR program is complete, briefly centrifuge the tubes.

2.3 WTA RPE PCR cleanup and quantification

Summary:

- RPE PCR cleanup (2 rounds)
- Quantify using Qubit™ Fluorometer

Preparation list:

Item		BD Part Number	Preparation & Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit™ dsDNA HS Assay Kit				
Obtain:				
RPE PCR product				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

It is recommended that different cleanup strategies are used for different quality sample types. Samples with lower quality or lower mRNA content may require more stringent cleanup methods.

Sample types	RPE PCR cleanup strategies	RPE PCR cleanup ratios	Cleanup details
High quality nuclei (nuclei isolated from high viability fresh samples, such as cell line and fresh PBMC, etc.)	Less stringent	2 rounds of 1.2x cleanup	1. 220 µL products + 264 µL beads* 2. 40 µL products + 60 µL NF water* + 120 µL beads
Low quality nuclei (nuclei isolated from previously frozen sample or low viability cells)	More stringent	1.0x cleanup +0.8x cleanup	1. 220 µL products + 220 µL beads* 2. 40 µL products + 60 µL NF water* + 80 µL beads

*beads: AMPure® XP beads, NF water: nuclease-free water

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume of 80% ethyl alcohol depending on the number of samples. One sample requires 2 mL 80% ethyl alcohol.

3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the RPE PCR product.
5. Combine the **four** tubes of **61 µL** RPE PCR into a new 1.5-mL LoBind® tube.
6. Pipet-mix 10 times.
7. Transfer exactly **220 µL** RPE PCR product to a new 1.5-mL LoBind® tube.
8. Pipet **220 µL/264 µL** of AMPure (**1.0x/1.2x**) into the tube.
9. Pipet-mix 10 times.
10. Briefly centrifuge the tube.

    Avoid getting AMPure® XP beads on the lid of the tube. Residual AMPure® XP beads and PCR mix buffer can negatively impact downstream results.

11. Incubate at room temperature for **5 minutes**.
12. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
13. Remove and discard the supernatant.
14. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
15. Incubate for **30 seconds**.

16. Remove and discard the supernatant without disturbing the beads.
17. Repeat steps 14–16 once for a total of **two ethyl alcohol washes**.
18. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
19. Air-dry the beads at room temperature until the beads no longer look glossy (**~3 minutes**)

    Do not overdry the AMPure® XP beads after the ethyl alcohol washes. Overdried beads appear cracked.

20. Remove the tube from the magnet.
21. Pipet **40 µL** of elution buffer into the tube.
22. Pipet-mix 10 times until the beads are fully resuspended.
23. Incubate at room temperature for **2 minutes**.
24. Briefly centrifuge the tube.
25. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
26. Pipet the eluate (40 µL) into a new 0.2-mL PCR strip tube.
27. Add **60 µL** of water to the eluate for a final volume of **100 µL**.

    The volume must be exactly 100 µL.

28. Pipet **80 µL/120 µL** of AMPure® XP beads (**0.8x/1.2x**) into the tube.
29. Pipet-mix 10 times.
30. Briefly centrifuge the tube.
31. Incubate at room temperature for **5 minutes**.
32. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
33. Remove and discard the supernatant.
34. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
35. Incubate for **30 seconds**.
36. Remove and discard the supernatant without disturbing the beads.
37. Repeat steps 34–36 for a total of **two ethyl alcohol washes**.
38. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
39. Air-dry the beads at room temperature until the beads no longer look glossy (**~3 minutes**).
40. Remove the tube from the magnet.
41. Pipet **30 µL** of elution buffer into the tube.
42. Pipet-mix 10 times until the beads are fully resuspended.

43. Incubate at room temperature for **2 minutes**.
44. Briefly centrifuge the tube.
45. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
46. Pipet the eluate (**30 µL**) into a new 1.5-mL LoBind® tube.

The purified RPE PCR product is ready for WTA index PCR (page 63).

Quantify the RPE PCR products with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay.

STOP The RPE PCR libraries can be stored at –20 °C for up to 6 months.

2.4 WTA index PCR

Summary:

- Prepare WTA index PCR mix
- Amplify using WTA index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes before setting up WTA Index PCR. Centrifuge briefly. Keep on ice until ready.	–20 °C
	WTA reverse primer 1–8	Various		
	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with WTA index PCR program				

Procedure steps:

This section describes how to generate mRNA libraries compatible with various sequencing platforms, by adding full-length sequencing adapters and indices through PCR. We provide reagents for unique dual-indexing, with different library forward primers and reverse primers for up to 8 samples.

Consult sequencing platform guidelines for low-plex pooling, to ensure the indices chosen meet the color balancing guidelines for the sequencing instrument that will be used.

1. In a new 1.5-mL LoBind® tube, pipet the following components.

WTA index PCR Mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
	PCR master mix	12.5	15.0	60.0	120.0
	Forward primer 1–8	2.5	3.0	N/A	N/A
	WTA reverse primer 1–8	2.5	3.0	N/A	N/A
	Nuclease-free water	22.5	27.0	108.0	216.0
	Total	**40.0**	**48.0**	**168.0**	**336.0**

2. Pipet-mix the WTA index PCR mix.
3. For multiple samples, pipet **35 µL** into separate 0.2-mL PCR tubes for each sample.
4. Add **2.5 µL** of forward primer and **2.5 µL** of reverse primer to each sample

   If index primers have already been added to the PCR mix, i.e. for 1 library, they do not need to be added again.

5. Place on ice until ready to use.
6. Dilute an aliquot of the purified RPE PCR product from step 46 of WTA RPE PCR cleanup and quantification (page 59) with water to **0.5 ng/µL**.

   If RPE PCR product concentration is <0.5 ng/µL, adjust the number of index PCR cycles as outlined in the table.

7. Add **10 µL** of RPE PCR product to **40 µL** index PCR mix.
8. Pipet-mix 10 times.

9. Run the following PCR program.

WTA index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	RPE PCR concentration* < 0.2 ng/µL: 13 cycles 0.2 ng/µL: 12 cycles 0.5 ng/µL: 10 cycles	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

*Recommended number of PCR cycles might require optimization for different sample types.

STOP The PCR can run overnight.

10. When the WTA index PCR program is complete, briefly centrifuge the tubes.

2.5 WTA index PCR cleanup and quality check

Summary:

- WTA index PCR cleanup
- Quality check using Qubit™ Fluorometer and BioAnalyzer/TapeStation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
	Nuclease-free water	51-9025552		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit™ dsDNA HS Assay Kit				
Agilent Bioanalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
WTA index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

This section describes how to perform a single-sided AMPure® XP beads cleanup for sequencing. The final product is purified double-stranded DNA with full-length adapter sequences.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours. Adjust the volume of 80% ethyl alcohol depending on the number of samples – one sample requires 0.5 mL 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Add **60 µL** of water to **50 µL** of the WTA index PCR product.
5. Transfer **100 µL** of WTA index PCR product into a new 0.2-mL PCR tube.

   The volume must be exactly 100 µL.

6. Pipet **80 µL** of AMPure® XP beads (**0.8x**) into the tube.
7. Pipet-mix 10 times.
8. Briefly centrifuge the tube.
9. Incubate at room temperature for **5 minutes**.
10. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
11. Remove and discard the supernatant.
12. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
13. Incubate for **30 seconds**.
14. Remove and discard the supernatant without disturbing the beads.
15. Repeat steps 12–14 once for a total of **two ethyl alcohol washes**.
16. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
17. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).
18. Remove the tube from the magnet.
19. Pipet **30 µL** of elution buffer into the tube.
20. Pipet-mix 10 times until the beads are fully resuspended.
21. Incubate at room temperature for **2 minutes**.
22. Briefly centrifuge the tube.
23. Place the tube on the magnet until the solution is clear (**~30 seconds**).
24. Pipet the eluate (**30 µL**) into a new 1.5-mL LoBind® tube.

The purified eluate is the final sequencing library.

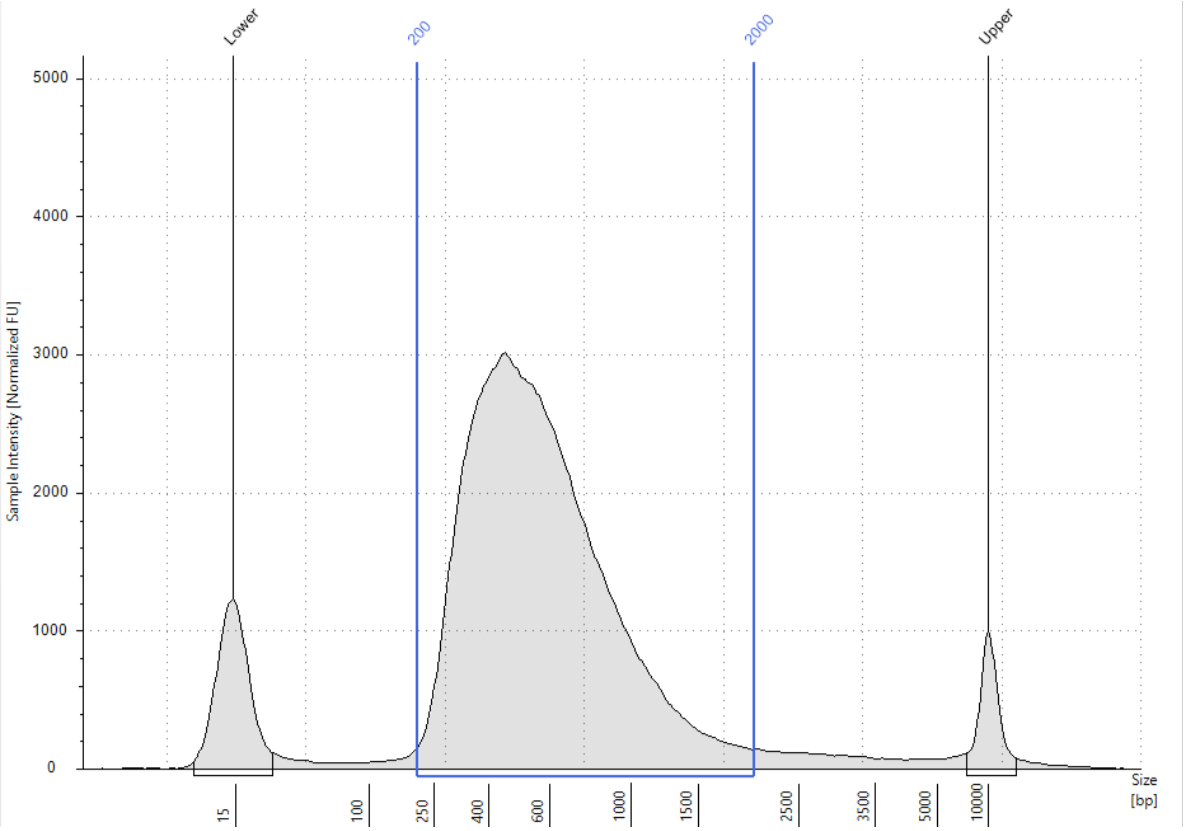
STOP The index PCR libraries can be stored at –20 °C for up to 6 months until sequencing.

25. Quantify and perform quality control of the WTA index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:
    - Agilent 2100 BioAnalyzer using the Agilent High Sensitivity DNA Kit
    - Agilent 4200 TapeStation system using the Agilent High Sensitivity D5000 ScreenTape assay

The expected concentration from the Qubit™ Fluorometer is **>1 ng/µL**.

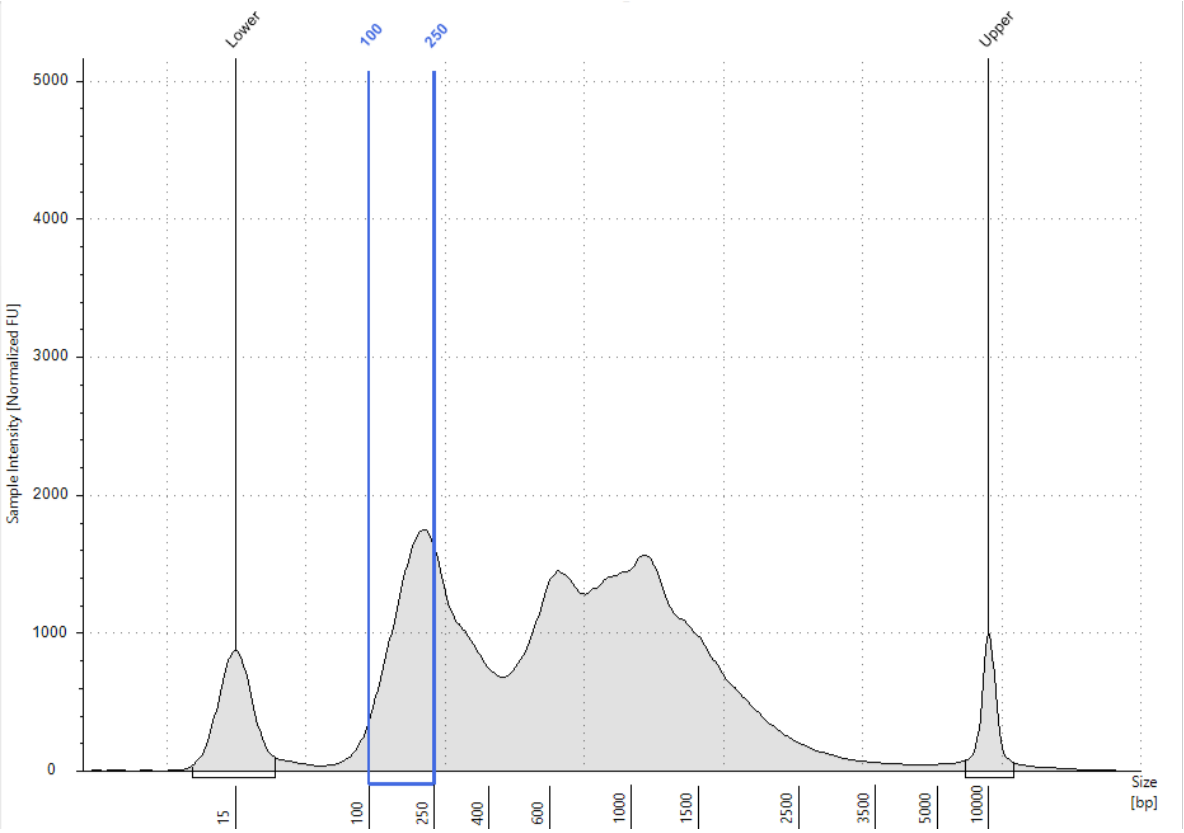
The TapeStation trace should show a peak from ~200 to 2,000 bp. Refer to the representative traces in the following figures.

Figure 2 Representative TapeStation High Sensitivity D5000 trace – WTA index PCR product



If smaller products (< 250 bp) are observed (such as the peaks shown in Figure 3), we recommend a second round of AMPure® XP bead purification. See Additional WTA index PCR cleanup (page 70) for more information.

Figure 3 Representative TapeStation High Sensitivity D5000 trace for WTA index PCR product with an observable noise peak in the smaller fragment region



2.6 Additional WTA index PCR cleanup

1. To the eluate from step 24 (page 67) in WTA index PCR cleanup and quality check (page 66), bring up the total volume to **100 µL** with water.
2. Pipet-mix 10 times.
3. Briefly centrifuge the tube.

   The volume must be exactly 100 µL.

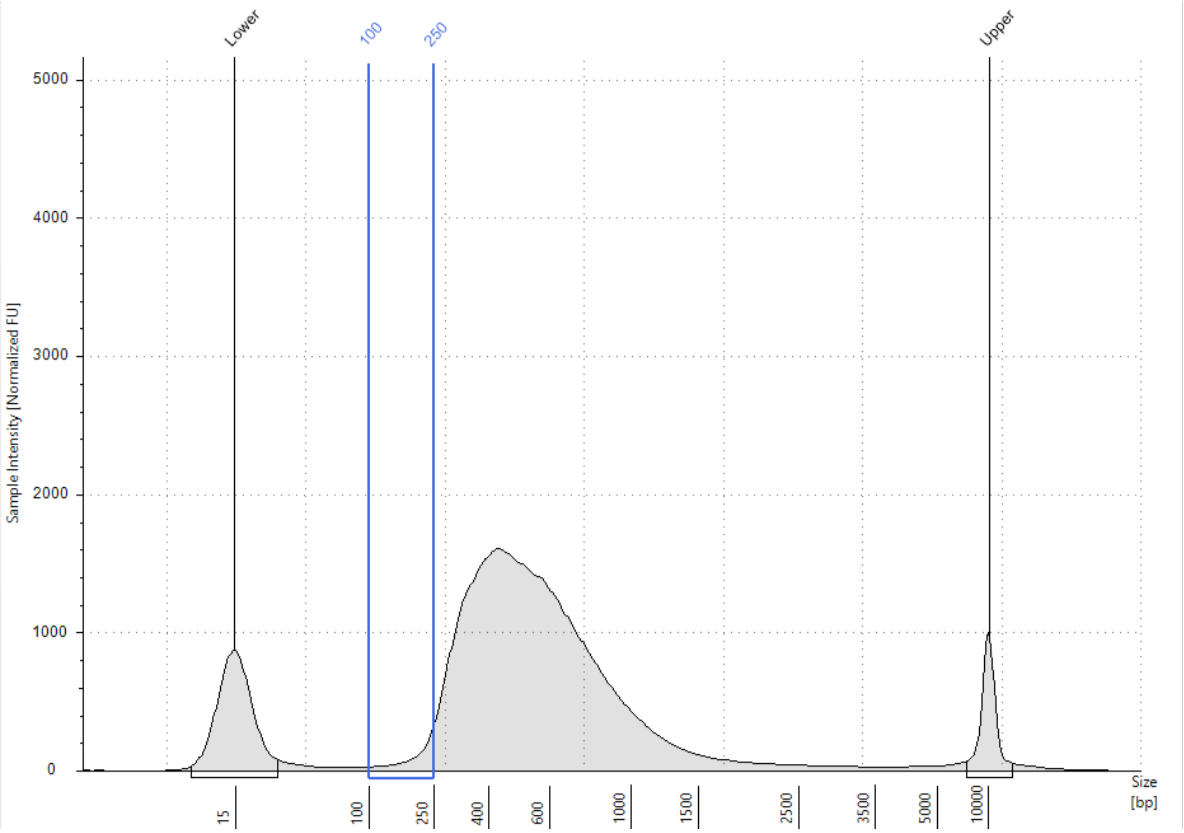
4. Pipet **80 µL** of AMPure® XP beads (**0.8x**) into the tube containing 100 µL sample.
5. Pipet-mix 10 times.
6. Briefly centrifuge the tube.
7. Incubate at room temperature for **5 minutes**.
8. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
9. Remove and discard the supernatant.
10. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
11. Incubate for **30 seconds**.
12. Remove and discard the supernatant without disturbing the beads.
13. Repeat steps 10–12 once for a total of **two ethyl alcohol washes**.
14. Keeping the tube on the magnet, use a small-volume pipette to remove and discard any residual supernatant from the tube.
15. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).
16. Remove the tube from the magnet.
17. Pipet **30 µL** of elution buffer into the tube.
18. Pipet-mix 10 times until the beads are fully resuspended.
19. Incubate at room temperature for **2 minutes**.
20. Briefly centrifuge the tube.
21. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
22. Pipet the eluate (30 µL) into a new 1.5-mL LoBind® tube.

    The purified eluate is the final sequencing library.

23. Repeat step 25 (page 68) in WTA index PCR cleanup and quality check (page 66) to perform quality check of the final libraries.

STOP The index PCR libraries can be stored at –20 °C for up to 6 months until sequencing.

Figure 4 Representative TapeStation High Sensitivity D5000 trace for WTA index PCR product after removal of noise peak in the smaller fragment region



3. Sample Tag library amplification

Before you begin:

- Obtain beads from step 25 of WTA Random Priming and Extension (RPE) (page 53).
- Thaw reagents (except for the enzymes) in the BD OMICS-One™ WTA Next Amplification Kit at room temperature (15–25 °C), then immediately place on ice. Keep enzymes at –25 °C to –15 °C.

3.1 Sample Tag PCR1

Summary:

- Prepare Sample Tag PCR1 mix
- Amplify using Sample Tag PCR1 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟢	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up Sample Tag PCR1. Centrifuge briefly. Keep on ice until ready.	–20 °C
🔴	Sample Tag PCR1 primer	51-9025470		
⚪	Nuclease-free water	51-9025552		
Place on ice:				
🟡	Bead RT/PCR enhancer	51-9025495	Centrifuge briefly before adding to mix.	–20 °C
Leave in freezer until ready to use:				
🟢	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with Sample Tag PCR1 program				

Procedure steps:

Please dilute universal oligo with nuclease-free water 4 fold to use in sample tag PCR1.

1. In the pre-amplification workspace, in a new 1.5-mL tube, pipet the following components.

Sample Tag PCR1 reaction mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
🟢	PCR master mix	50.0	60.0	240.0	480.0
🟢	4x diluted universal oligo	1.2	1.44	5.76	11.52
🟡	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
🔴	Sample Tag PCR1 primer	1.2	1.44	5.76	11.52
⚪	Nuclease-free water	135.6	162.72	650.88	1,301.76
	Total	**200**	**240**	**960**	**1920**

2. Gently vortex the mix, briefly centrifuge, and place back on ice.
3. Place the tube of RPE-treated beads from step 25 of WTA Random Priming and Extension (RPE) (page 53) on 1.5 mL magnet for <2 minutes.
4. Remove and discard the supernatant.
5. Remove the tube from the magnet, and resuspend the beads in a **200 µL** Sample Tag PCR1 reaction mix. Do not vortex.
6. Ensuring that the beads are fully resuspended, pipet **50 µL** PCR1 reaction mix with the beads into each of the **four** 0.2-mL PCR tubes. Transfer any residual mix to one of the tubes.
7. Bring the reaction mix to the post-amplification workspace.
8. Program the thermal cycler. Do not use fast cycling mode.

Sample Tag PCR1 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C[a]	45 seconds
Denaturation	9–13[b]	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

a. To avoid beads settling due to prolonged incubation time on thermal cycler before the denaturation step, it is critical to pause the instrument at 98 °C before loading the samples. Different thermal cyclers might have different pause time settings. In certain brands of thermal cyclers, however, we have observed a step-skipping error with the pause/unpause functions. To ensure that the full 45-second denaturation is not skipped, verify that the pause/unpause functions are working correctly on your thermal cycler. To avoid the step-skipping problem, a one-minute 98 °C pause step can be added immediately before the 45-second 98 °C denaturation step.

b. Suggested PCR cycles might need to be optimized for different cell types and cell number.

Suggested number of PCR cycles

Number of nuclei in PCR1	Suggested PCR cycles for resting PBMCs
1,000	13
2,500	12
5,000	11
10,000	10
20,000	9

9. Ramp heated lid and heat block of post-amplification thermal cycler to 98 °C by starting the thermal cycler program and then pausing it.

   Do not proceed to thermal cycling until each tube is gently mixed by pipette to ensure uniform bead suspension.

10. For each 0.2-mL PCR tube, gently pipet-mix, immediately place tube in thermal cycler, and unpause the thermal cycler program.

    STOP The PCR can run overnight but proceed with purification within 24 hours after PCR.

11. After PCR, briefly centrifuge tubes.
12. Pipet-mix and combine the four reactions into a new 1.5-mL LoBind® tube.

    Retain the supernatant in the next step.

13. Place the 1.5-mL tube on magnet for 2 minutes, and carefully pipet the supernatant (Sample Tag PCR1 products) into the new 1.5 mL LoBind® tube without disturbing the beads.

    Discard the BD Rhapsody™ Enhanced Cell Capture Beads after use.

3.2 Sample Tag PCR1 cleanup

Summary:

- Sample tag PCR1 cleanup

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
AMPure® XP magnetic beads			Manufacturer's recommendations	
Obtain:				
Sample Tag PCR1 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

Perform the purification in the post-amplification space.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume of 80% ethyl alcohol depending on the number of samples – one sample requires 1 mL 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the Sample Tag PCR1 product.

   The volume must be exactly 200 µL. If the volume is less than 200 µL, use water to achieve the final volume.
5. Pipet **280 µL** of AMPure® XP beads (**1.4x**) into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.

   Avoid getting AMPure® XP beads on the lid of the tube. Residual AMPure® XP beads and PCR mix buffer can negatively impact downstream results.
8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for **30 seconds**.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 13–15 once for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy (**~3 minutes**).

    Do not overdry AMPure® XP beads after ethyl alcohol washes. Overdried beads appear cracked.
17. Remove the tube from the magnet.
18. Pipet **30 µL** of elution buffer into the tube.
19. Pipet-mix 10 times until the beads are fully resuspended.
20. Incubate at room temperature for **2 minutes**.
21. Briefly centrifuge the tube.

22. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
23. Pipet the eluate (**30 µL**) into a new 1.5-mL tube.

    The purified Sample Tag PCR1 product is ready for Sample Tag PCR2.
24. Use 5 µL undiluted Sample Tag PCR1 product for Sample Tag PCR2 (page 79)

STOP The Sample Tag PCR1 libraries can be stored at –20 °C for up to 6 months.

3.3 Sample Tag PCR2

Summary:

- Prepare Sample Tag PCR2 mix
- Amplify using Sample Tag PCR2 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟢	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up Sample Tag PCR2. Centrifuge briefly. Keep on ice until ready.	–20 °C
🔴	Sample Tag PCR2 primer	51-9025471		
⚪	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
🟢	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with Sample Tag PCR2 program				

Procedure steps:

Please dilute universal oligo with nuclease-free water 4 fold to use in sample tag PCR2.

1. In the pre-amplification workspace, in a new 1.5-mL tube, pipet the following components.

Sample Tag PCR2 reaction mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
●	PCR master mix	12.5	15.0	60.0	120.0
●	4x diluted universal oligo	2.0	2.4	9.6	19.2
●	Sample Tag PCR2 primer	3.0	3.6	14.4	28.8
○	Nuclease-free water	27.5	33.0	132.0	264.0
	Total	**45.0**	**54.0**	**216.0**	**432.0**

2. Place on ice till ready to use.
3. Pipet **45 µL** of Sample Tag PCR2 mix into a 0.2-mL PCR tube for each sample.
4. Bring the tubes to the post-amplification workspace.
5. Add **5 µL** undiluted Sample Tag PCR1 product.
6. Pipet-mix 10 times.
7. Run the following PCR program.

Sample Tag PCR2 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	10[a]	98 °C	15 seconds
Annealing		66 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

a. Recommended number of PCR cycles might require optimization for different cell types.

STOP The PCR can run overnight.

3.4 Sample Tag PCR2 cleanup and quantification

Summary:

- Sample Tag PCR2 cleanup
- Quantify using Qubit™ Fluorometer

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit™ dsDNA HS Assay Kit				
Obtain:				
Sample Tag PCR2 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

Perform PCR2 purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume of 80% ethyl alcohol depending on the number of samples. One sample requires 0.5 mL 80% ethyl alcohol.

3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the Sample Tag PCR2 product.

   The volume must be exactly **50 µL**. If the volume is less than 50 µL, use water to achieve the final volume.

5. Pipet **60 µL** of AMPure® XP beads (**1.2x**) into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.

   Avoid getting AMPure® XP beads on the lid of the tube. Residual AMPure® XP beads and PCR mix buffer can negatively impact downstream results.

8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for **30 seconds**.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 11–13 once for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).

    Do not overdry AMPure® XP beads after ethyl alcohol washes. Overdried beads appear cracked.

17. Remove the tube from the magnet.
18. Pipet **30 µL** of elution buffer into the tube.
19. Pipet-mix 10 times until the beads are fully resuspended.
20. Incubate at room temperature for **2 minutes**.

21. Briefly centrifuge the tube.
22. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
23. Pipet the eluate (**30 µL**) into a new 1.5-mL tube.

    The purified Sample Tag PCR2 product is ready for Sample Tag index PCR (page 84)
24. Quantify the PCR2 products with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay.
25. Dilute an aliquot of the PCR2 products with nuclease-free water to 0.1–1.1 ng/µL.

STOP The Sample Tag PCR2 libraries can be stored at –20 °C for up to 6 months.

3.5 Sample Tag index PCR

Summary:

- Prepare Sample Tag index PCR mix
- Amplify using Sample Tag index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes before setting up Sample Tag index PCR. Centrifuge briefly. Keep on ice until ready.	–20 °C
○	Multiomic reverse primer 1–8	Various		
○	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with Sample Tag index PCR program				

Procedure steps:

1. In the pre-amplification workspace, pipet the following reagents into a new 1.5-mL LoBind® tube on ice.

Sample Tag index PCR Mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
●	PCR master mix	12.5	15.0	60.0	120.0
●	Forward primer 1–8	2.0	2.4	9.6	19.2
○	Multiomic reverse primer 1–8[a]	2.0	2.4	NA	NA
○	Nuclease-free water	30.5	36.6	146.4	292.8
	Total	**47.0**	**56.4**	**216.0**	**432.0**

a. For more than one library, use different library reverse primers for each library.

2. Gently vortex mix, briefly centrifuge, and place back on ice.
3. In a new 0.2-mL PCR tube, combine Sample Tag index PCR mix with diluted Sample Tag PCR2 products as follows:
   - For one sample, add **47 µL** of Sample Tag index PCR mix to the PCR tube in which **3 µL** of 0.1–1.1 ng/uL Sample Tag PCR2 products will be added in post-amplification workspace.
   - If working with multiple samples, combine **45 µL** of Sample Tag index PCR mix with **2 µL** of corresponding library reverse primer to the PCR tube in which **3 µL** of 0.1–1.1 ng/uL Sample Tag PCR2 products will be added in post-amplifcation workspace.
4. Bring index PCR mixes into the post-amplification workspace.
5. Pipet 3.0 µL of 0.1–1.1 ng/µL Sample Tag PCR2 products into 47.0 µL index PCR mix.
6. Gently vortex, and briefly centrifuge.
7. Program the thermal cycler. Do not use fast cycling mode.

Sample Tag index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	6–8[a]	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	30 seconds
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

a. Suggested PCR cycles.

Suggested number of PCR cycles

Concentration index PCR input for Sample Tag libraries (ng/µL)	Suggested PCR cycles
0.5–1.1	6
0.25–0.5	7
0.1–0.25	8

STOP The PCR can run overnight.

3.6 Sample Tag index PCR cleanup and quality check

Summary:

- Sample Tag index PCR cleanup
- Quality check using Qubit™ Fluorometer and Bioanalyzer/TapeStation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
	Nuclease-free water	51-9025552		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit™ dsDNA HS Assay Kit				
Agilent Bioanalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
Sample Tag index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

Perform purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume of 80% ethyl alcohol depending on the number of samples – one sample requires 0.5 mL 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with Sample Tag index PCR product.

   The volume must be exactly **50 µL**. If the volume is less than 50 µL, use water to achieve the final volume.
5. Pipet **40 µL** of AMPure® XP beads (**0.8x**) into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.
8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for 30 seconds.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 11–13 once for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).
17. Remove the tube from the magnet.
18. Pipet **30 µL** of elution buffer into the tube.
19. Pipet-mix 10 times until the beads are fully resuspended.
20. Incubate at room temperature for **2 minutes**.
21. Briefly centrifuge the tube.
22. Place the tube on the magnet until the supernatant is clear (**~30 seconds**).
23. Pipet the eluate (**30 µL**) into a new 1.5-mL LoBind® tube.

    The purified eluate is the final sequencing library.

STOP The index PCR libraries can be stored at –20°C for up to 6 months until sequencing.

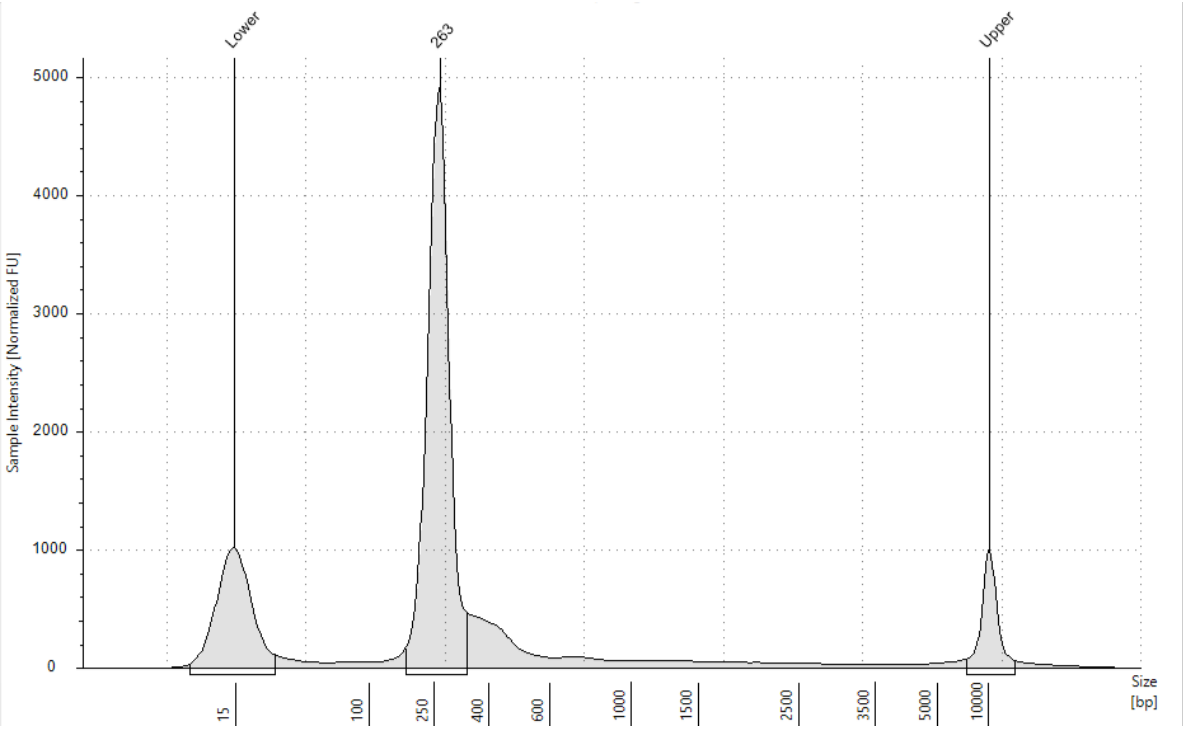
24. Quantify and perform quality control of the Sample Tag index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:
    - Agilent 2100 BioAnalyzer using the Agilent High Sensitivity DNA Kit
    - Agilent 4200 TapeStation system using the Agilent High Sensitivity D1000 or D5000 ScreenTape assay

    The Sample Tag library should show a peak of ~276 bp.*

    * Exact size might vary due to instrument or sample purification efficiency. Select the region size between 200 and 500 bp.

Sample Tag indexed product

Figure 5 Sample TapeStation high-sensitivity D5000 trace



Sequencing

ATAC library requirements

- Recommended sequencing depth: 50,000 read pairs per cell.

  Required parameters:

Parameter	Requirement
Platform	Illumina® and Element
Paired-end reads	Recommended: Read 1: 50 cycles; Read 2: 50 cycles Index 1: 8 cycles; Index 2: 60 cycles
PhiX	1% recommended
Analysis	See the *BD Rhapsody™ Sequence Analysis Pipeline User's Guide* (Doc ID: 23-24580)

Reverse primer sequences

ATAC Library Reverse Primer	Sequences
1	TAAGGCGA
2	CGTACTAG
3	AGGCAGAA
4	TCCTGAGC
5	GGACTCCT
6	TAGGCATG
7	CTCTCTAC
8	CAGAGAGG

ATAC library sequencing recommendations

- For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration in the range of 1.5–1.8 pM with 1% PhiX spike-in.
- For other sequencing platforms (e.g. Element AVITI System), follow the manufacturer's sequencing recommendations.

WTA and Sample Tag library requirements

Required parameters:

Parameter	Requirement
Platform	Illumina® and Element
Paired-end reads	Recommend: Read 1: 51 cycles; Read 2: 71 cycles
PhiX	1% recommended
Analysis	See the *BD Rhapsody™ Sequence Analysis Pipeline User's Guide* (Doc ID: 23-24580)

Reverse primer sequences

WTA Library Reverse Primer	Multiomic Library Reverse Primer for Sample Tag libraries	Sequences
1	1	GCGTAGTA
2	2	CGGAGCCT
3	3	TACGCTGC
4	4	ATGCGCAG
5	5	TAGCGCTC
6	6	ACTGAGCG
7	7	CCTAAGAC
8	8	CGATCAGT

WTA and Sample Tag library sequencing recommendations

- For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration in the range 1.5–1.8 pM with 1% PhiX for a sequencing run.
- For other sequencing platforms (e.g. Element AVITI System), follow the manufacturer's sequencing recommendations.
- Sequencing depth for WTA can vary depending on whether the sample contains high- or low-content RNA cells. For resting PBMCs, the reads-per-cell recommendations are as follows:
  - 10,000 reads per cell for shallow sequencing. Genes per cell and UMI per cell detected is generally lower but can be useful for cell type identification.
  - 50,000 reads per cell for moderate sequencing.
  - 100,000 reads per cell for deep sequencing to harvest the majority of UMIs in the library.
  - For sample tag, we recommend 2000 reads per cell.

WTA and Sample Tag libraries can be pooled together with ATAC libraries to sequence following ATAC sequencing recommendations. Please be aware that only reserve index will be used for demultiplexing samples. Contact your local Field Application Specialist (FAS) or scomix@bd.com for compatible sequencer information.

Single-cell ATAC-Seq WTA library sequencing analysis pipeline

Contact your local Field Application Specialist (FAS) or scomix@bd.com for access to the latest BD Rhapsody™ sequence analysis pipeline.

Appendix

Our ATAC kits are configured for use with the BD Rhapsody™ HT Xpress System. For BD Rhapsody™ Express System users, contact your local Field Application Specialist (FAS) or us at scomix@bd.com, if RNase inhibitor or Proteinase K quantities prove insufficient.

Rhapsody single-lane cartridge workflow (no scan other than indicated)

1. Prime the cartridge.
   a. Prior to priming the Rhapsody™ cartridge, scan the empty cartridge for Cell Load scan. For detailed instructions, see *BD Rhapsody™ System - Single-Cell Capture and cDNA Synthesis with BD Rhapsody Single-Cell Analysis System* (Doc ID: 23-22951).
   b. Place waste collection container in the BD Rhapsody™ Express System.
   c. Prime the cartridge using the following table steps with a BD Rhapsody™ P1200M pipette.

Step number	Material to load	Volume (µL)	Pipette mode	Incubation at room temperature
1	100% ethyl alcohol	700	Prime/Treat	N/A
2	Air	700	Prime/Treat	N/A
3	Room temp. Cartridge Wash Buffer 1	700	Prime/Treat	1 min
4	Air	700	Prime/Treat	N/A
5	Room temp. Cartridge Wash Buffer 1	700	Prime/Treat	10 min
6	Air	700	Prime/Treat	N/A
7	Room temp. Cartridge Wash Buffer 2	700	Prime/Treat	≤4 hr

2. Prepare single-nuclei suspension for cartridge loading:

   Use the BD Rhapsody™ Scanner to calculate the number of nuclei for cartridge loading.

   a. Use the Samples Calculator on the scanner to calculate the volumes of tagmented nuclei and modified sample buffer with RNase inhibitor needed to prepare a nuclei suspension of 650 µL (this volume is for one cartridge).
   b. Select the correct cartridge type. For the BD Rhapsody™ single-lane cartridge, use 0119.
   c. Prepare 650 µL nuclei suspension for cartridge loading by mixing unstained tagmented nuclei with cold modified sample buffer with RNase inhibitor according to the displayed volumes on the scanner. Ensure the stock solution of each sample is well suspended by gently pipet-mixing with a wide-bore tip before pooling. Keep the nuclei suspension on ice.
3. Load tagmented nuclei in the cartridge:

a. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Prime/Treat
• Set the BD Rhapsody™ P1200M pipette to **Cell Load** mode. • Pipet-mix the cell suspension using a manual P1000 pipette		
Nuclei suspension	575	Cell Load

Press button to aspirate 40 µL air, and then immerse tip in cell suspension. Press button again to aspirate 575 µL of cold nuclei 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.

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

4. Wash the loaded nuclei with cold sample buffer:

Do not omit this step. It is necessary to obtain good ATAC data.

a. Place the cartridge on the BD Rhapsody™ Express System.

b. Set the BD Rhapsody™ P1200M pipette to **Prime/Treat** mode.

c. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Prime/Treat
Cold sample buffer	700	Prime/Treat
Air	700	Prime/Treat
Cold sample buffer	700	Prime/Treat

5. Load and wash BD Rhapsody™ Enhanced Cell Capture Beads:

a. Place the cartridge on the BD Rhapsody™ Express System.

b. Set the BD Rhapsody™ P1200M pipette to **Prime/Treat** mode.

c. Bring the splint beads generated from the Splint bead generation step. Add another 370 µL sample buffer to increase the bead suspension volume to 750 µL.

d. Load the cartridge with materials listed below using the BD Rhapsody™ P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Prime/Treat
• Gently pipet-mix to completely resuspend the beads. • Set the BD Rhapsody™ P1200M pipette to **Bead Load** mode. • Immediately load the beads. Check the pipette tips to make sure that there are no air bubbles inside the tips before loading.		
Splint Beads	630	Bead Load

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

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

g. Once Bead Load is completed, tap **OK**, then **Eject**. Remove the cartridge from the scanner.

h. Place the cartridge on the BD Rhapsody™ Express System.

i. Set the BD Rhapsody™ P1200M pipette to **Wash mode**.

j. Load the cartridge with materials listed below using the BD Rhapsody™ P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Air	700	Wash
Cold sample buffer	700	Wash
Air	700	Wash
Cold sample buffer	700	Wash

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

6. Lyse nuclei:

a. Add 75.0 µL of 1 M DTT to one room temperature 15-mL lysis buffer bottle and briefly vortex mix. Use the lysis buffer with DTT within 24 hours, and then discard.

b. Pipette 1 mL of lysis buffer with DTT into a new 1.5-mL LoBind® tube. Add 50 µL of Proteinase K to the tube immediately before the lysis step, and gently pipet-mix 5 times.

c. Set the BD Rhapsody™ P1200M pipette to **Lysis** mode.

d. Load the cartridge with materials listed using the BD Rhapsody™ P1200M pipette:

Material to load	Volume (µL)	Pipette mode
Lysis buffer with DTT and Proteinase K	550	Lysis

e. Carefully remove the cartridge from the BD Rhapsody™ Express System. Slowly transfer the cartridge into

an incubator at 37 °C and incubate for 10 minutes. Maintain the recommended lysis time for best performance.

It is important to keep the cartidge leveled.

7. Retrieve cell-capture beads:
   a. Place the 5-mL LoBind tube into the BD Rhapsody™ Express System drawer. Label the tubes appropriately.
   b. Ensure that the BD Rhapsody™ P5000M pipette is set to **RETRIEVAL** mode.
   c. Move the front slider to **BEADS** on the BD Rhapsody™ Express System.
   d. Carefully bring the cartridge from the 37 °C incubator to the BD Rhapsody™ Express System and allow the cartridge to cool down for 5 minutes.
   e. Move the left slider to **RETRIEVAL.**
   f. Leave the retrieval magnet in the down position for 30 seconds.
   g. Aspirate 5,000 µL lysis buffer with DTT using the BD Rhapsody™ P5000M pipette.
   h. Press down on the BD Rhapsody™ P5000M pipette to seal against the gasket.
   i. Move the left slider to the middle position (**0**), and immediately load 4,950 µL of lysis buffer with DTT.
   j. Remove the pipette from the gasket and purge the tips.
   k. 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.
   l. Immediately proceed to Washing BD Rhapsody™ Enhanced Cell Capture Beads in the following section.
   m. Appropriately dispose of the cartridge, waste collection container, and lysis buffer with DTT.
8. Washing BD Rhapsody™ Enhanced Cell Capture Beads:
   a. 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.
   b. Remove the tube from the magnet. Gently pipet-mix the beads and transfer them to a new 1.5-mL LoBind tube.
   c. 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.
   d. Place the tube on a magnet for 2 minutes.
   e. Remove and discard the supernatant.
   f. Resuspend the beads in 1.0 mL lysis buffer with DTT.
   g. Incubate the tube in thermomixer at **37 °C**, 1200 rpm for **15 minutes**.
   h. Place the tube on a magnet rack for 2 minutes.
   i. Remove and discard the supernatant. Avoid leaving lysis buffer or bubbles in the tube. Otherwise, the lysis buffer might cause the ligation to fail.

j. Remove the tube from the magnet and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix.

k. Place the tube on the magnet rack for 2 minutes. Remove and discard the supernatant.

l. Remove the tube from the magnet, and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix, and place on ice.

Start ligation ≤30 minutes after washing retrieved cell-capture beads with bead wash buffer.

m. Proceed to Ligation (page 38).

Tagmentation reaction scaling up and down table

Scaling up to 100,000 nuclei

Cap	Kit Component	Volume for 100,000 nuclei (µL)
●	Tagmentation buffer with DMF	50
○	Nuclease-free water	23.5
●	10X PBS	4
●	RNase inhibitor	2.5
●	Digitonin 1%[a]	1
●	Tween 20, 10%	1
●	Tagmentase	8
	Nuclei	10
Total volume		**100**

a. Predilution with nuclease-free water before adding into the reaction mix.

Tagmentation buffer with DMF can only supply 500,000 nuclei reaction in total

Scaling down 5,000, 10,000, and 25,000 nuclei

Cap	Kit Component	Volume for 5,000 nuclei (µL)	Volume for 10,000 nuclei (µL)	Volume for 25,000 nuclei (µL)
●	Tagmentation buffer with DMF	5	5	12.5
○	Nuclease-free water	0	0	2.25
●	10X PBS	0.8 (2x pre-diluted[a])	0.8 (2x pre-diluted)	1
●	RNase Inhibitor	0.5 (2x pre-diluted)	0.5 (2x pre-diluted)	1.25 (2x pre-diluted)
●	Digitonin 1%[a]	0.5 (5x pre-diluted)	0.5 (5x pre-diluted)	0.5 (2x pre-diluted)
●	Tween 20, 10%	0.5 (5x pre-diluted)	0.5 (5x pre-diluted)	0.5 (2x pre-diluted)
●	Tagmentase	0.8	0.8	2
	Nuclei	2 (2,500 nuclei/µL)	2 (5,000 nuclei/µL)	5 (5,000 nuclei/µL)
Total volume		10.1	10.1	25

a. Predilution with nuclease-free water before adding into the reaction mix.

Optional manual cell load analysis

Total number of nuclei captured = number of nuclei counted/ number of wells × 227,000. At least 100 nuclei should be counted and also number of wells were counted in the following example.

Region	Nuclei count	No. of wells
1	26	225
2	14	225
3	17	225
4	22	225
5	26	225
Total	**105**	**1,125**

105/1,125×227,000 = 21,187

Contact information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

BD Biosciences
European Customer Support
Tel +32.53.720.600
help.biosciences@bd.com

bdbiosciences.com
scomix@bd.com