

 **BD** Rhapsody™ System
Nuclei Isolation
Protocol

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Regulatory information

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Disclaimer

The nuclei isolation protocols described herein have been adapted from the OMNI-ATAC workflow* and optimized to ensure compatibility with the BD Rhapsody™ Single Cell Analysis System and BD Rhapsody™ ATAC-Seq assays. Validation has been performed exclusively on human PBMCs, K562, Jurkat, and Ramos cell lines, as well as nuclei isolated from mouse brain, kidney, liver, heart, and pancreas tissues. While these protocols may be suitable for additional cell types or tissues, we strongly recommend that users evaluate, modify, or seek alternative protocols as appropriate for their specific sample type of interest to ensure optimal experimental outcomes.

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History

Revision	Date	Change made
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Introduction

This protocol is for nuclei isolation on the BD Rhapsody™ system.

Symbols

The following symbols are used in this guide:

Symbol	Description
	Caution: Hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death

Protocol I: BD Rhapsody™ nuclei isolation protocol for tissue samples

Required reagents and recommended materials

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

Required reagents

Materials	Supplier	Catalog no.
Iodixanol (optiprep)	Sigma-Aldrich	D1556-250ML
Sucrose	Sigma-Aldrich	S7903-250G
IGEPAL® CA-630	Sigma-Aldrich	I8896-100ML
Tween20	Sigma-Aldrich	P9416
0.5M EDTA, pH 8.0	Sigma-Aldrich	03690-100ML
β-mercaptoethanol	Sigma-Aldrich	M6250-100ML
PMSF	Sigma-Aldrich	P7626-1G
1M CaCl ₂	Sigma-Aldrich	21115-100ML
1M Mg(Ac) ₂	Sigma-Aldrich	63052-100ML
1M Tris pH 7.8	Sigma-Aldrich	T2569-1L
1M Tris-HCl, pH 7.4	Sigma-Aldrich	T2194-1L
5M NaCl	Sigma-Aldrich	S5150
1M MgCl ₂	Sigma-Aldrich	M1028
2-Propanol	Sigma-Aldrich	I9516-500ML
RNase inhibitor	New England Biolabs	M0314L

Materials	Supplier	Catalog no.
0.1M DTT	Thermo Fisher Scientific	707265ML
Nuclease-free water	Major supplier	–
Trypan blue	Major supplier	–

Recommended consumables

Supplies	Supplier	Catalog no.
Low retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
Wide-orifice, low retention LTS Tips, 200 µL	Major supplier	–
Wide-orifice, low retention LTS Tips, 1000 µL	Major supplier	–
INCYTO disposable hemocytometer	INCYTO	CN DHC-N01-5
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
Lint-free cloth (Kimwipes®)	Major supplier	–
0.2 Micron disposable filter units with PES membrane	Major supplier	–
Cell strainer, pore size 100 µm	MilliporeSigma	CLS431752-50EA

Required equipment

Equipment	Supplier	Catalog no.
2-mL Dounce tissue grinder set	Sigma-Aldrich	D8938-1SET
Temperature-controlled centrifuge	Major supplier	–
Cell counter	Major supplier	–

Before you begin

Prepare the following buffer, sterile filter the buffer, and store at room temperature.

6X Homogenization buffer stable master mix

Reagent	Final concentration	Volume for 100 mL
1M CaCl ₂	30 mM	3.0 mL
1M Mg(Ac) ₂	18 mM	1.8 mL
1M Tris pH 7.8	60 mM	6.0 mL
Nuclease-free water	–	89.2 mL

1M Sucrose

Reagent	Materials needed
Sucrose	34.23 g
Nuclease-free water	78.5 mL

Prepare the following solutions, sterile filter the buffer, and store at 4 °C.

10% IGEPAL® CA-630

Reagent	Materials needed
IGEPAL® CA-630	1.0 mL
Nuclease-free water	9.0 mL

10% Tween20

Reagent	Materials needed
Tween20	1.0 mL
Nuclease-free water	9.0 mL

Prepare 100 mM PMSF solution and store at –20 °C.

PMSF

Reagent	Materials needed
PMSF	34.8 mg
2-Propanol	2.0 mL



PMSF is harmful to the mucosa of the respiratory tract, eyes, and skin and can be lethal if inhaled, ingested, or absorbed through the skin. In case of eye or skin contact with PMSF, rinse immediately with plenty of water. Clothing contaminated with PMSF should be discarded.

Prepare the following buffers on the day of processing. Put on ice until use.

Nuclei wash buffer

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
1M Tris-HCl, pH 7.4	10 mM	150	180
5M NaCl	10 mM	30	36
1M MgCl ₂	3 mM	45	54
10% Tween20	0.1%	150	180
Nuclease-free water	–	14,625	17,550

6X Homogenization buffer unstable solution (650 μL per sample)

Reagent	Final concentration	Volume per sample (μL)
6X Homogenization buffer stable	6X	648.84
100 mM PMSF	0.1 mM	1.08
14.3M β-mercaptoethanol	1 mM	0.08

50% Iodixanol solution (400 μL per sample)

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
6X Homogenization buffer unstable	1X	66.67	80.00
60% Iodixanol solution (optiprep)	50%	333.33	400.00

For BD Rhapsody™ single-cell ATAC-seq, prepare the following buffer solutions without RNase inhibitor.

1X Homogenization buffer unstable solution (1 mL per sample)

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
6X Homogenization buffer unstable	1X	166.67	200
1M Sucrose	320 mM	320.00	384
0.5M EDTA, pH 8.0	0.1 mM	0.20	0.24
10% IGEPAL® CA-630	0.1%	10.00	12
Nuclease-free water	–	503.13	603.76

29% Iodixanol solution (600 µL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
6X Homogenization buffer unstable	1X	100.00	120.00
1M Sucrose	160 mM	96.00	115.20
60% Iodixanol solution (optiprep)	29%	290.00	348.00
Nuclease-free water	–	114.00	136.8

35% Iodixanol solution (600 µL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
6X Homogenization buffer unstable	1X	100.00	120.00
1M Sucrose	160 mM	96.00	115.20
60% Iodixanol solution (optiprep)	35%	350.00	420.00
Nuclease-free water	–	54.00	64.8

For BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis, add RNase inhibitor in the following buffer solutions to preserve RNA quality.

1X Homogenization buffer unstable solution (1 mL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
6X Homogenization buffer unstable	1X	166.67	200.00
1M Sucrose	320 mM	320.00	384.00
0.5M EDTA, pH 8.0	0.1 mM	0.20	0.24
10% IGEPAL® CA-630	0.1%	10.00	12.00
RNase inhibitor	2 U/µL	50.00	60.00
100 mM DTT	1 mM	10.00	12.00
Nuclease-free water	–	443.13	531.76

29% Iodixanol solution (600 µL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
6X Homogenization buffer unstable	1X	100.00	120.00
1M Sucrose	160 mM	96.00	115.20

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
60% Iodixanol solution (optiprep)	29%	290.00	348.00
RNase inhibitor	1 U/μL	15.00	18.00
100 mM DTT	1 mM	6.00	7.20
Nuclease-free water	–	93.00	111.60

35% Iodixanol solution (600 μL per sample)

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
6X Homogenization buffer unstable	1X	100.00	120.00
1M Sucrose	160 mM	96.00	115.20
60% Iodixanol solution (optiprep)	35%	350.00	420.00
RNase inhibitor	1 U/μL	15.00	18.00
100 mM DTT	1 mM	6.00	7.20
Nuclease-free water	–	33.00	39.60

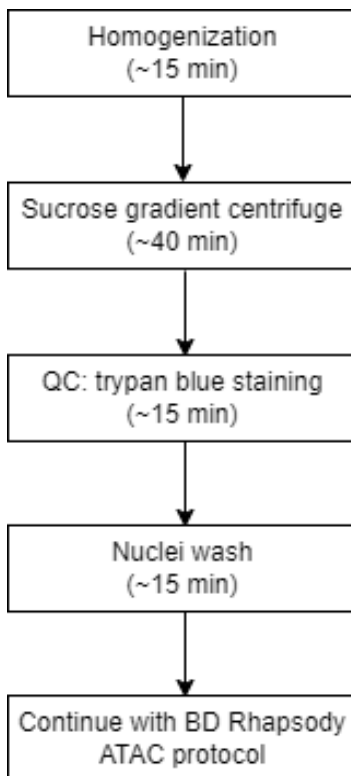
Nuclei buffer with RNase inhibitor (25 μL per sample)

Reagent	Final concentration	Volume (μL)
Nuclei buffer*	–	193
RNase inhibitor	1 U/μL	5
0.1M DTT	1 mM	2
*Use the nuclei buffer from BD Rhapsody™ Tagmentation and Supplemental Reagents Kit		

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.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells and nuclei.

Time considerations



Procedure

All steps should be performed on ice or at 4 °C. Pre-chill a swinging-bucket centrifuge to 4 °C.

1. Obtain frozen tissue sample and put it on dry ice. It is recommended to use tissue size of ~50 mg.
2. Transfer the frozen tissue piece into a pre-chilled 2-mL Dounce tissue grinder and add 1 mL of cold 1X homogenization buffer unstable solution.
3. Allow the frozen tissue to thaw on ice for 5 minutes. Often the tissue chunk will sink to the bottom of the Dounce tissue grinder once thawed.
4. Gently homogenize the tissue with the A pestle until resistance goes away (~ 10-20 strokes).
5. Pre-clear by filtration through the 100 µm cell strainer filter.
6. Gently homogenize the tissue with the B pestle until resistance goes away (~ 20-30 strokes).
7. Centrifuge at 100g for 1 minute at 4 °C.
8. Transfer 400 µL supernatant into a new 2.0 mL Lo-Bind eppendorf tube.
9. Add 1 volume (400 µL) of 50% Iodixanol solution to give a final concentration of 25% Iodixanol. Mix by pipetting 10 times.
10. Layer 600 µL of 29% Iodixanol solution under the 25% mixture. Avoid mixing of layers.
11. Layer 600 µL of 35% Iodixanol solution under the 29% mixture. Avoid mixing of layers.

Note: This step requires gradual removal of the pipette tip during pipetting to avoid excessive volume displacement.
12. Centrifuge the tube at 3000g for 20 minutes at 4°C in a swinging bucket centrifuge with the brake off.
13. Aspirate the top layers down to within 300 µL of the nuclei band.
 - a. For some tissue types, floating cell debris may be present. Use a 1.0 mL wide-bore tip to remove them.
 - b. Nuclei layer may be at different locations for different tissues. The following volumes may need adjustment based on your tissue type.
 - Top layer ~700 µL
 - Middle layer ~300–400 µL
 - Nuclei layer ~200–400 µL
14. Using 200 µL volume, collect the nuclei band and transfer to a new 1.5-mL Lo-Bind tube.
15. Count the nuclei with trypan blue staining. Mix 10 µL trypan blue with 10 µL nuclei solution and count nuclei using cell counter. The trypan blue positive rate should be larger than 90%.
16. Transfer ~500,000 nuclei into a new 15 mL tube, add 10 mL nuclei wash buffer and mix by inverting the tube 5 times.
17. Centrifuge at 500g for 5 minutes at 4°C.
18. Discard supernatant and resuspend the nuclei pellet in 5 mL nuclei wash buffer.
19. Centrifuge at 500g for 5 minutes at 4°C.

- 20. Discard supernatant and resuspend the nuclei pellet in 25 μ L nuclei buffer (or modified nuclei buffer for BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis).
- 21. Continue with Step “Prepare nuclei suspension for tagmentation” in *BD Rhapsody™ Single-Cell ATAC-Seq (Doc ID: 23-24473)* or *BD Rhapsody™ Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis (Doc ID: 23-24474)*.

Protocol II: BD Rhapsody™ nuclei isolation protocol for cell line samples

Required reagents and recommended materials

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

Required reagents

Materials	Supplier	Catalog no.
1M Tris-HCl, pH 7.4	Sigma-Aldrich	T2194-1L
5M NaCl	Sigma-Aldrich	S5150
1M MgCl ₂	Sigma-Aldrich	M1028
IGEPAL® CA-630	Sigma-Aldrich	I8896-100ML
Tween20	Sigma-Aldrich	P9416
RNase inhibitor	New England Biolabs	M0314L
0.1M DTT	Thermo Fisher Scientific	707265ML
2% Digitonin	Promega	G9441
Gibco™ DPBS, no calcium, no magnesium	Thermo Fisher Scientific	14-190-144
Nuclease-free water	Major supplier	–
Trypan blue	Major supplier	–

Recommended consumables

Supplies	Supplier	Catalog no.
Low retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
Wide-orifice, low retention LTS Tips, 200 µL	Major supplier	–
INCYTO disposable hemocytometer	INCYTO	CN DHC-N01-5
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
Lint-free cloth (Kimwipes®)	Major supplier	–
0.2 Micron disposable filter unit with PES membrane	Major supplier	–

Required equipment

Equipment	Supplier	Catalog no.
Temperature-controlled centrifuge	Major supplier	–
Cell counter	Major supplier	–

Before you begin

Prepare the following solutions, sterile filter the buffer, and store at 4 °C.

10% IGEPAL® CA-630

Reagent	Materials needed
IGEPAL® CA-630	1.0 mL
Nuclease-free water	9.0 mL

10% Tween20

Reagent	Materials needed
Tween20	1.0 mL
Nuclease-free water	9.0 mL

Prepare the following buffers on the day of processing. Put on ice until use.

Nuclei wash buffer (5 mL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
1M Tris-HCl, pH 7.4	10 mM	50	60
5M NaCl	10 mM	10	12
1M MgCl ₂	3 mM	15	18
10% Tween20	0.1%	50	60
Nuclease-free water	–	4875	5850

For BD Rhapsody™ single-cell ATAC-seq, prepare the following buffer solutions without RNase inhibitor.

Nuclei lysis buffer (500 µL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
1M Tris-HCl, pH 7.4	10 mM	5	6
5M NaCl	10 mM	1	1.2

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
1M MgCl ₂	3 mM	1.5	1.8
10% IGEPAL® CA-630	0.1%	5	6
10% Tween20	0.1%	5	6
2% Digitonin	0.01%	2.5	3
Nuclease-free water	–	480	576

For BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis, add RNase inhibitor in the following buffer solutions to preserve RNA quality.

Nuclei lysis buffer with RNase inhibitor (500 μL per sample)

Reagent	Final concentration	Volume per sample (μL)	1 sample with 20% overage (μL)
1M Tris-HCl, pH 7.4	10 mM	5	6
5M NaCl	10 mM	1	1.2
1M MgCl ₂	3 mM	1.5	1.8
10% IGEPAL® CA-630	0.1%	5	6
10% Tween20	0.1%	5	6
2% Digitonin	0.01%	2.5	3
RNase inhibitor	1 U/μL	12.5	15
Nuclease-free water	–	467.5	561

Nuclei buffer with RNase inhibitor (25 μL per sample)

Reagent	Final concentration	Volume (μL)
Nuclei buffer*	–	193
RNase inhibitor	1 U/μL	5
0.1M DTT	1 mM	2
*Use the nuclei buffer from BD Rhapsody™ Tagmentation and Supplemental Reagents Kit		

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.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells and nuclei.

Procedure

Pre-chill a swinging-bucket centrifuge to 4 °C.

1. Confirm that the cell viability is > 90% with trypan blue staining prior to proceeding to the next step.
2. Transfer 500K cells into a 15-mL tube.
3. Centrifuge the cells at 500g for 5 minutes at 4 °C.
4. Carefully remove the tube from the centrifuge and remove and discard the supernatant without disturbing the cell pellet.
5. Resuspend the cell pellet with 9 mL 1X PBS without Ca/Mg.
6. Centrifuge the cells at 500g for 5 minutes at 4 °C.
7. Carefully remove the tube from the centrifuge and completely remove the supernatant without disturbing the cell pellet.
8. On ice, add 500 µL of freshly prepared Nuclei Lysis Buffer (or Nuclei Lysis Buffer with RNase inhibitor for BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis) into the tube.
9. Resuspend the cells by gentle pipetting up and down 5 times, and then incubate on ice for 5 minutes.
10. Add 5 mL of freshly prepared Nuclei Wash Buffer into the tube.
11. Mix by inverting the tube 5 times.
12. Centrifuge the tube at 500g for 3 minutes at 4 °C.
13. Carefully remove the tube from the centrifuge and discard the supernatant without disturbing the nuclei pellet.

Note: Some residual buffer may be left to avoid removing the nuclei pellet.

14. Add 25 µL of nuclei buffer (or nuclei buffer with RNase inhibitor for BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis).
15. Resuspend the nuclei pellet by gentle pipetting 10 times with a wide bore tip. Keep on ice.
16. Continue with Step “Prepare nuclei suspension for tagmentation” in *BD Rhapsody™ Single-Cell ATAC-Seq (Doc ID: 23-24473)* or *BD Rhapsody™ Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis (Doc ID: 23-24474)*.

Protocol III: BD Rhapsody™ nuclei isolation protocol for cryopreserved PBMCs

Required reagents and recommended materials

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

Required reagents

Materials	Supplier	Catalog no.
1M Tris-HCl, pH 7.4	Sigma-Aldrich	T2194-1L
5M NaCl	Sigma-Aldrich	S5150
1M MgCl ₂	Sigma-Aldrich	M1028
IGEPAL® CA-630	Sigma-Aldrich	I8896-100ML
Tween20	Sigma-Aldrich	P9416
RNase inhibitor	New England Biolabs	M0314L
0.1M DTT	Thermo Fisher Scientific	707265ML
2% Digitonin	Promega	G9441
Gibco™ DPBS, no calcium, no magnesium	Thermo Fisher Scientific	14-190-144
Bovine serum albumin (BSA)	Sigma-Aldrich	126609
RPMI 1640 medium	Thermo Fisher Scientific	11875093
Fetal bovine serum (FBS), premium, heat-inactivated	Thermo Fisher Scientific	A5670801
Antibiotic-Antimycotic (100X)	Thermo Fisher Scientific	15240062
Calcein AM*	Thermo Fisher Scientific	V35004
DRAQ7*	Thermo Fisher Scientific	564904
Nuclease-free water	Major supplier	–
Trypan blue	Major supplier	–
* Protect Calcein AM and DRAQ7 from light. Avoid multiple freeze-thaw cycles. See manufacturer's storage recommendations.		

Recommended consumables

Supplies	Supplier	Catalog no.
Low retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–

Supplies	Supplier	Catalog no.
Wide-orifice, low retention LTS tips, 200 µL	Major supplier	–
INCYTO disposable hemocytometer	INCYTO	CN DHC-N01-5
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
Lint-free cloth (Kimwipes®)	Major supplier	–
0.2 Micron disposable filter unit with PES membrane	Major supplier	–

Required equipment

Equipment	Supplier	Catalog no.
BD Rhapsody™ Scanner*	BD Biosciences	633701
Temperature-controlled centrifuge	Major supplier	–
Cell counter	Major supplier	–

* Part of the BD Rhapsody™ HT Single-Cell Analysis System. Items can be ordered separately.

Before you begin

Prepare the following solutions, sterile filter the buffer, and store at 4°C.

10% IGEPAL® CA-630

Reagent	Materials needed
IGEPAL® CA-630	1.0 mL
Nuclease-free water	9.0 mL

10% Tween20

Reagent	Materials needed
Tween20	1.0 mL
Nuclease-free water	9.0 mL

10% BSA

Reagent	Materials needed
BSA	1.0 g
Nuclease-free water	10.0 mL

RPMI medium with 10% FBS and 1X antibiotics

Reagent	Final concentration
RPMI 1640 Medium	–
Fetal bovine serum (FBS), premium, heat-inactivated	10%
Antibiotic-Antimycotic (100X)	1X

Pre-warm in 37 °C water bath for 30 minutes before use.

Prepare the following buffers on the day of processing. Put on ice until use.

Nuclei wash buffer (5 mL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
1M Tris-HCl, pH 7.4	10 mM	50	60
5M NaCl	10 mM	10	12
1M MgCl ₂	3 mM	15	18
10% Tween20	0.1%	50	60
Nuclease-free water	–	4875	5850

PBS with 0.04% BSA

Reagent	Materials needed
1X PBS without calcium & magnesium	10.0 mL
10% BSA	40 µL

For BD Rhapsody™ single-cell ATAC-seq, prepare the following buffer solutions without RNase inhibitor.

Nuclei lysis buffer (500 µL per sample)

Reagent	Final concentration	Volume per sample (µL)	1 sample with 20% overage (µL)
1M Tris-HCl, pH 7.4	10 mM	5	6
5M NaCl	10 mM	1	1.2
1M MgCl ₂	3 mM	1.5	1.8
10% IGEPAL® CA-630	0.1%	5	6
10% Tween20	0.1%	5	6
2% Digitonin	0.01%	2.5	3
Nuclease-free water	–	480	576

For BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis, add RNase inhibitor in the following buffer solutions to preserve RNA quality.

Nuclei lysis buffer with RNase inhibitor (500 μ L per sample)

Reagent	Final concentration	Volume per sample (μ L)	1 sample with 20% overage (μ L)
1M Tris-HCl, pH 7.4	10 mM	5	6
5M NaCl	10 mM	1	1.2
1M MgCl ₂	3 mM	1.5	1.8
10% IGEPAL® CA-630	0.1%	5	6
10% Tween20	0.1%	5	6
2% Digitonin	0.01%	2.5	3
RNase inhibitor	1 U/ μ L	12.5	15
Nuclease-free water	–	467.5	561

Nuclei buffer with RNase inhibitor (25 μ L per sample)

Reagent	Final concentration	Volume (μ L)
Nuclei buffer*	–	193
RNase inhibitor	1 U/ μ L	5
0.1M DTT	1 mM	2

*Use the nuclei buffer from BD Rhapsody™ Tagmentation and Supplemental Reagents Kit

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.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells and nuclei.

Procedure

Pre-chill a swinging-bucket centrifuge to 4 °C. Pre-warm RPMI medium with 10% FBS and 1X antibiotics in 37 °C water bath for 30 minutes before use.

1. Take cryopreserved PBMCs out of liquid nitrogen tank and quickly thaw the cells in the 37 °C water bath until ~80% thawed (~1.5 minutes).
2. Slowly add 1 mL of warm RPMI medium with 10% FBS and 1X antibiotics to the vial while agitating/swirling the tube. Gently pipet mix 5 times.
3. Transfer the cells into a 15-mL conical tube and add warm RPMI medium with 10% FBS and 1X antibiotics

to make a total volume of 10 mL.

- 4. Centrifuge the cells at 400g for 5 minutes at room temperature.
- 5. Carefully remove the tube from the centrifuge then remove the supernatant, but leave ~0.5 mL of residual supernatant without disturbing the cell pellet.
- 6. Add 0.5 mL of RPMI medium with 10% FBS and 1X antibiotics into the tube.
- 7. Tap the tube to break the pellet and then resuspend the cells by pipet-mixing 5 times.
- 8. Count the cells and check cell viability with trypan blue staining.
- 9. Centrifuge the cells in RPMI medium at 400g for 5 minutes at 4 °C.
- 10. Carefully remove the tube from the centrifuge and discard the supernatant.
- 11. Resuspend the cell pellet in 2 mL of PBS with 0.04% BSA. Gently pipet-mix 5 times.
- 12. Centrifuge the cells at 150g for 5 minutes at 4 °C. Carefully remove the tube from the centrifuge, then remove and discard the supernatant.
- 13. Repeat steps 11 and 12 for 2 more times for a total of 3 washes.
- 14. After the third wash, resuspend the cells in 1 mL PBS with 0.04% BSA by gently pipet-mixing 5 times. Keep the cells on ice.
- 15. Take an aliquot of the cells and dilute 10-fold with cold Sample Buffer for cell count.
 - a. Pipet 90 µL of cold Sample Buffer from BD Rhapsody™ Enhanced Cartridge Reagent Kit and transfer to a new 1.5-mL LoBind tube.
 - b. Ensure the cells are well suspended by gently pipet-mixing.
 - c. Pipet 10 µL of the mixed cell suspension and transfer into the tube with 90 µL cold Sample Buffer. Keep the remaining cells on ice.
 - d. Add 0.5 µL of Calcein AM and 0.5 µL of DRAQ7 into the tube.
 - e. Gently pipet-mix 10 times and incubate at 37 °C for 5 minutes away from light.
- 16. Count the stained cells immediately using the BD Rhapsody™ Scanner.
 - a. Ensure the cells are well suspended by gently pipet-mixing.
 - b. Pipet 10 µL into the INCYTO disposable hemocytometer and count by the scanner.
 - c. Multiply the reading by 10 for original cell concentration.
 - d. If cell viability > 90%, proceed to the next step.
- 17. Transfer 500K of the washed PBMCs to a 15-mL tube.
- 18. Centrifuge the cells at 500g for 5 minutes at 4 °C.
- 19. Carefully remove the tube from the centrifuge and discard the supernatant without disturbing the cell pellet.
- 20. On ice, add 500 µL of freshly prepared Nuclei Lysis Buffer (or Nuclei Lysis Buffer with RNase inhibitor for BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis) to the tube.
- 21. Resuspend the cells by gently pipet-mixing 5 times and incubate on ice for 3 minutes.

- 22. Add 5 mL of freshly prepared Nuclei Wash Buffer to the tube.
- 23. Mix by inverting the tube 5 times.
- 24. Centrifuge the tube at 500g for 5 minutes at 4 °C.
- 25. Carefully remove the tube from the centrifuge and discard the supernatant without disturbing the nuclei pellet.
- 26. Add 25 µL of nuclei buffer (or nuclei buffer with RNase inhibitor for BD Rhapsody™ single-cell ATAC-seq and mRNA Whole Transcriptome Analysis).
- 27. Resuspend the nuclei pellet by gentle pipetting 10 times with a wide bore tip. Keep on ice.
- 28. Continue with Step “Prepare nuclei suspension for tagmentation” in *BD Rhapsody™ Single-Cell ATAC-Seq (Doc ID: 23-24473)* or *BD Rhapsody™ Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis (Doc ID: 23-24474)*.

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