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Nuclei isolation from fresh frozen human colon tissue for 10X Genomics Multi-omics (ATAC + GEX) assay

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Protocol status: In development
It works, but we are still developing and optimizing this protocol

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Disclaimer

This protocol is still being optimized! At the moment, we are testing if we can achieve better results without using the collagenase/dispase as dispase is a proteinase.

The buffers are based on the user guides from 10X Genomics.

Abstract

Nuclei isolation protocol used for sn multi-omics assay on fresh frozen human colon tissue (full thickness and muscularis layer only).

Materials

BUFFERS

Buffers based on 10X Genomics user guides.

Collagenase/Dispase 10269638001 from Sigma

	A	B	C	D	E	F
	Collagenase Lysis Buffer	Stock conc		Final conc		V (µl)
	Tris-HCl pH 7.4	1000	mM	10	mM	10,5
	NaCl	5000	mM	10,0	mM	2,1
	MgCl2	1000	mM	3	mM	3,2
	DTT	1000	mM	1	mM	1,1
	RNAseIn	40	U/uL	1	U/uL	26,3
	Dispase/collagenase	0,1	mg/mL	0,003	mg/mL	31,5
	NF H2O					975,5
	Total Volume					1050,0
	Wash Buffer 1	Stock conc		Final conc		V (µl)
	Tris-HCl (pH 7.4)	1000	mM	10	mM	28,4
	NaCl	5000	mM	10	mM	5,7
	MgCl2	1000	mM	3	mM	8,5
	BSA	10	%	1	%	283,5
	Tween-20	10	%	0,1	%	28,4

	A	B	C	D	E	F
	DTT	1000	mM	1	mM	2,8
	RNase in plus (promega)	40	U/ul	1	U/ul	70,9
	Water					2406,9
	Total Volume					2835,0
	0.1x Lysis	Stock conc		Final conc		V (µl)
	Tris-HCl (pH 7.4)	1000	mM	10	mM	5,3
	NaCl	5000	mM	10	mM	1,1
	MgCl2	1000	mM	3	mM	1,6
	Tween-20	10	%	0,01	%	0,5
	NP-40	10	%	0,01	%	0,5
	Digitonin	0,5	%	0,002	%	2,1
	BSA	10	%	1	%	52,5
	DTT	1000	mM	1	mM	0,5
	Rnase Inhibitor Protector (SIGMA)	40	U/ul	1	U/ul	13,1
	Water					447,8
	Total Volume					525,0
	Wash Buffer 2	Stock conc		Final conc		V (µl)
	Tris-HCl (pH 7.4)	1000	mM	10	mM	10,5

	A	B	C	D	E	F
	NaCl	5000	mM	10	mM	2,1
	MgCl2	1000	mM	3	mM	3,2
	BSA	10	%	1	%	105,0
	Tween-20	10	%	0,1	%	10,5
	DTT	1000	mM	1	mM	1,1
	Rnase Inhibitor Protector (SIGMA)	40	U/ul	1	U/ul	26,3
	Water					891,5
	Total Volume					1050,0
	1X Diluted Nuclei buffer	Stock conc		Final conc		V(µl)
	Nuclei buffer	20	X	1	X	10
	DTT	100	mM	1	mM	2
	Rnase Inhibitor Protector (SIGMA)	40	U/ul	1	U/ul	5
	Water					183,0
	Total Volume					200

Instruments and equipment:
- Kai Medical 2 mm Biopsy punch BP-20F
- WPI Noyes scissors 12 cm S/S 15 mm blades
- VWR®, Disposable Pestles and Cordless Motor for Pellet Mixing
- PluriSelect 40 µm, 20 µm filters
- Flowmi 40 µm Cell strainer for 1000P
- Luna FL Cell Counter
- Swing out rotor centrifuge

Sample preparation

1 Place the sample preparation instruments on dry ice: biopsy punch, 1.5 mL EP tube, tweezers, petri dish.

2 Using 2 mm biopsy punch, aliquot 1.5-2 tissue pieces per patient. Place the pieces in a fresh 1.5 mL EP tube placed on dry ice. Repeat with a fresh biopsy punch for each multiplexed patient.

Experiment preparation

3 Prepare all buffers fresh and on wet ice. Add the the detergents, DTT and RNase inhibitor just before use.

4 Place all the plastics and filters on wet ice.

Tissue homogenization

5 **Perform all steps on wet ice.** Add 500 µL Collagenase Lysis Buffer to the tube with multiplexed patient samples. Let thaw slightly.

6 Cut the tissue with scissors until there are no more pieces visible. **Time =** around 4 min (depending on the amount and the characteristics of the tissue).

6.1 After cutting, place the scissors in a fresh 1.5 mL EP tube placed on ice.

7 Further homogenize the tissue using an automatic plastic pellet pestle, for 45 s.

7.1 Place the pellet pestle in the EP tube instead of the scissors.

8 Mince the tissue for 1 min.

9 Wash the scissors using 250 µL Collagenase Lysis Buffer (while adding it to the tissue).

10 Homogenize the tissue with the used pellet pestle x15 (by hand).

11 Wash the pestle with 250µL Collagenase Lysis Buffer and add it to the tissue.

12 Gently pipet up and down 10X with P1000.

13 Centrifugate for 5 min at 500g at 4°C, remove the supernatant and resuspend in 1 mL Wash Buffer 1.

14 With P1000, transfer the sample onto 40 and 20 µm stacked strainers placed on 25 mL EP tube.

15 Wash the 1.5 mL EP tube with additional 1 mL Wash buffer 1, transfer onto the top filter.

16 Centrifugate the 25 mL EP tube for 5 min at 500g, 4°C.

16.1 Gently remove the supernatant.

17 Resuspend the pellet in 700 µL of Wash Buffer 1 and filtrate through Flowmi filter into a fresh DNA LoBind 1.5 mL EP tube.

18 Centrifugate for 5 min at 500g at 4°C

18.1 Gently remove the supernatant.

Nuclei permeabilization

19 Resuspend the pellet in 500 µL 0.1x Lysis Buffer, pipette mix X5 with P1000.

20 Incubate on ice for 1 min (tissue quality dependent).

21 Add 1000 µL of Wash Buffer 2 and pipette mix 5X with P1000.

22 Centrifugate for 5 min at 500 g at 5°C.

22.1 Very gently remove the supernatant.

Nuclei resuspension and counting

23 Resuspend the pellet in 25-50 µL 1X Diluted Nuclei Buffer.

24 Cell count the nuclei and proceed to the 10X Genomics Chromium Next GEM Single Cell Multiome ATAC + Gene Expression assay.