JAX - Nuclei Isolation for 10x Genomics A V.1

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Protocol status: Working
We use this protocol and it's working

ABSTRACT

The purpose of this protocol is to produce single nuclei from frozen human tissues for downstream assaying with the 10x Genomics Multiome assay.

This protocol has been demonstrated using Human placenta tissue as well as human Glioblastoma.

This protocol is modified from Sigma Aldrich Nuclei Isolation Kit: EZ Prep protocol and 10x Chromium Demonstrated protocol for Single Cell Multiome ATAC + Gene Expression Sequencing (CG000365).

GUIDELINES

1. Assess nuclear membrane integrity (refer to 10x Genomics CG000365), using Brightfield or Trypan blue
2. Assess for debris, if large amount of debris present, filter with smaller pore size to decrease debris.
3. Use either Trypan blue and Countess (for clean average size nuclei preps) or AO/PI and Nexcellom K2 counter (if nuclei prep has debris)
4. If RNA content is of concern Imaging nuclei can be done with fluorescence, and Syto RNA stain

MATERIALS

Nuclei Buffer (20x): 2000153/2000207 (10x Genomics)
Digitonin: BN2006 (Thermo Fisher)
Trizma Hydrochloride Solution, pH 7.4: T2194 (Sigma Aldrich)
Magnesium Chloride Solution: M1028 (Sigma Aldrich)
Sodium Chloride solution, 5M: 59222C (Sigma Aldrich)
1M Nonidet P40 substitute:74385(Sigma Aldrich)
Protector RNase inhibitor (DO NOT SUBSTITUTE): 3335399001 (Sigma Aldrich)
Nuclei Isolation Kit: Nuclei EZ Prep: NUC101-1KT (Sigma Aldrich)
DTT: 43816-10ML (Sigma Aldrich)

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Tween-20: 1662404 (Bio-Rad)
PBS: 14190-144(Gibco)
BSA: 130-091-376 (Miltenyi)
Countess Slides: C10228 (Invitrogen)
Flowmi 40um filter (Bel-Art™ H136800040)
Bel-art disposable pestle: BAF199230000-100EA (Sigma Aldrich)
Pluristrainer mini filters: 43-10040-40 (Pluriselect)

Buffers

A	B	C	D
	Stock	Final	1mL
EZ lysis	-	-	972ul
Digitonin	5%	0.01%	2ul
DTT	1000 mM	1 mM	1 µl
RNase inhibitor 40 U/µl	40 U/µl	1 U/µl	25ul

Lysis Buffer, Prepare fresh, maintain at 4°C

A	B	C	D
	Stock	Final	4mL
Tris-HCl (pH 7.4)	1 M	10 mM	40 µl
NaCl	5 M	10 mM	8 µl
MgCl2	1 M	3 mM	12 µl
Tween-20	10%	1.00%	400 µl
BSA	10%	0%	40 µl
DTT	1000 mM	1 mM	4 µl
RNase inhibitor 40 U/µl	40 U/µl	1 U/µl	100 µl
Nuclease-free Water	-	-	3.40 ml

Wash Buffer, Prepare fresh, maintain at 4°C

A	B	C	D
	Stock	Final	1mL
Nuclei Buffer* (20X)	20X	1X	50 µl

	A	B	C	D
	DTT	1000 mM	1mM	1 µl
	RNase inhibitor	40 U/µl	1 U/µl	25 µl
	Nuclease-free Water	-	-	924 µl

Nuclei Buffer (1X), Prepare fresh, maintain at 4°C

BEFORE START INSTRUCTIONS

Familiarize yourself with 10x Genomics protocol CG000365.

Sample Prep

20m

1 Slightly thaw sample out on ice and place onto pre chilled Petri dish on ice.

2 Cut tissue into small pieces and add to a microcentrifuge tube.

Cell lysis

17m

3 In micro centrifuge tube containing the tissue add 100 µL lysis buffer of lysis buffer.

4 Using a plastic pestle to grind tissue by pushing pestle down and twisting to break up the tissue. Start timer once lysis buffer is added. 2m

5 Add 25 µL lysis buffer to wash off pestle and place tube On ice for a total of 00:05:00. 5m

6 After 5 minutes of lysis On ice , 500 x g, 4°C, 00:05:00 . 5m

7 Remove supernatant and resuspend pellet using wide bore tips in 100 µL lysis buffer .

8 Incubate On ice for a total of 00:05:00 . 5m

Washing 15m

9 Add 500 µL wash buffer and 500 x g, 4°C, 00:05:00 . 5m

10 go to step #9 Repeat for 3 total washes. 10m

11 After last wash, filter suspension through 40um filter.

12 Resuspend sample in 1x Nuclei buffer (10x provided) containing RNase inhibitor, and DTT.

Load 5m

13 Load for transposition and continue with the 10x multiome protocol.