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Nuclei isolation and permeabilisation of fresh frozen human brain samples for 10X Genomics Multiome

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We use this protocol and it's working

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Abstract

This protocol details the procedure of nuclei isolation and debris removal from fresh frozen brain tissue in preparation for the 10X Genomics multiome ATAC/GEX assay.

Materials

HOW MANY SAMPLES TO PROCESS? 2

	A	B	C	D	E	F
	2X salt-Tris solution	Stock conc		Final conc		V (µl)
	NaCl	5000	mM	292	mM	1226,4
	Tris pH7.5	1000	mM	20	mM	420,0
	CaCl	1000	mM	2	mM	42,0
	MgCl2	1000	mM	42	mM	882,0
	Water					18429,6
	Total Volume					21000,0

	TST+NP Homogenization buffer	Stock conc		Final conc		V (µl)
	2X salt-Tris solution	2	X	1	X	1100,0
	Tween-20	10	%	0,03	%	6,6
	NP-40	10	%	0,03	%	6,6
	BSA	7,5	%	1	%	293,3
	KCl	3000	mM	25	mM	18,3
	Sucrose	2000	mM	250	mM	275,0

	DTT	1000	mM	1	mM	2,2
	cOmplete protease inhibitor	50	X	1	X	44,0
	Rnase In plus (promega)	40	U/ul	1	U/ul	55,0
	Water					398,9
	Total Volume					2200,0

	Wash buffer 1	Stock conc		Final conc		V (µl)
	2X salt-Tris solution	2	X	1	X	561,0
	BSA	7,5	%	1	%	149,6
	KCl	3000	mM	25	mM	9,4
	Sucrose	2000	mM	250	mM	140,3
	DTT	1000	mM	1	mM	1,1
	cOmplete protease inhibitor	50	X	1	X	22,4
	Rnase In plus (promega)	40	U/ul	1	U/ul	28,1
	Water					210,2
	Total Volume					1122,0

	GM (Gradient Medium)	Stock conc		Final conc		V(µl)

	CaCl	1000	mM	1	mM	1,3
	Optiprep	60	%	50	%	1083,3
	MgCl2	1000	mM	5	mM	6,5
	Tris pH 7.5	1000	mM	10	mM	13,0
	Sucrose	2000	mM	75	mM	48,8
	DTT	1000	mM	1	mM	1,3
	cOmplete protease inhibitor	50	X	0,5	X	13,0
	Rnase In plus (promega)	40	U/ul	1	U/ul	32,5
	Water					100,3
	Total Volume					1300

	ODM (Optiprep Diluent Medium)	Stock conc		Final conc		V(µl)
	KCl	3000	mM	150	mM	62,0
	MgCl2	1000	mM	30	mM	37,2
	Tris pH 8	1000	mM	60	mM	74,4
	Sucrose	2000	mM	250	mM	155,0
	RNAse in plus (promega)	40	U/uL	0,5	U/uL	15,5
	Water					895,9

	Total Volume					1240,0

	29% Cushion	Stock conc	Final conc	V(µl)		
	Optiprep	60	%	29	%	966,7
	ODM					1033,3
	Total Volume					2000

	1x Lysis	Stock conc		Final conc		V (µl)
	Tris-HCl (pH 7.4)	1000	mM	100	mM	50,0
	NaCl	5000	mM	100	mM	10,0
	MgCl2	1000	mM	30	mM	15,0
	Tween-20	10	%	0,1	%	5,0
	NP-40	10	%	0,1	%	5,0
	Digitonin	5	%	0,02	%	2,0
	Water					413,0
	Total Volume					500,0

	0.1x Lysis	Stock conc		Final conc		V (µl)
	1x lysis	1	x	0,1	x	100,0

	BSA	7,5	%	1	%	133,3
	DTT	1000	mM	1	mM	1,0
	Rnase Inhibitor (Promega)	40	U/ul	1	U/ul	25,0
	Water					740,7
	Total Volume					1000,0

	Wash Buffer 2	Stock conc		Final conc		V (µl)
	Tris-HCl (pH 7.4)	1000	mM	10	mM	22,0
	NaCl	5000	mM	10	mM	4,4
	MgCl2	1000	mM	3	mM	6,6
	BSA	7,5	%	1	%	293,3
	Tween-20	10	%	0,01	%	2,2
	DTT	1000	mM	1	mM	2,2
	Rnase Inhibitor (SIGMA)	40	U/ul	1	U/ul	55,0
	Water					1814,3
	Total Volume					2200,0

	1X Diluted Nuclei buffer	Stock conc		Final conc	V(µl)	

	Nuclei buffer	20	X	1	X	7,5
	DTT	100	mM	1	mM	1,5
	Rnase Inhibitor (SIGMA)	40	U/ul	1	U/ul	3,75
	Water					137,25
	Total Volume					150

Preparation

1 Prepare the 2X salt-Tris solution and aliquot to 1 ml tube and freeze at -20°C

2 Prepare the BSA in PBS and filter through 0.2um filter.

3 Prepare the Tween-20 Homogenization lysis buffer fresh for each preperations

4 Filter sucrose 2M solution through 0.2um/0.45um filter

5 Prepare cOmplete protease inhibitor (50X) by dissolving a tablet in 1 mL of water.

6 Filter protease inhibitor solution through 0.2um filter

7 Place the homogenizer at -80°C at the start and leave 10 min before homgenization on ice.

Homogenization (Peform all steps on ice)

8 Cut brain piece on dry ice (if a large section), then transfer immediately in homogenizer containing 250 uL of TST+NP Homogenization Lysis Buffer. Immediately add 500uL TST+NP Homogenization Lysis Buffer

9 Allow the tissue to thaw for 2 mins in the homogenization buffer.

10 Homogenize tissue with pestle A (10X), wait 1min, and with pestle B (10X).

11 Put the homogenate through 70µm cell strainer and place the falcon on ice. Incubate on ice for 5 mins. Rinse dounce and filter with 250uL TST+NP HB.

12 During the incubation (in about 4 mins), use LUNA-fl counter to assess the viability of the nuclei.

13 Transfer the contents from the 50 mL falcon to a 2 mL protein lo-bind tube.

14 Centrifuge @ 500xg for 5 mins. Discard the supernatant.

15 Resuspend the pellet in 200 uL of Wash Buffer 1, and transfer to a 2mL DNA lobind tube.

16 Add additional 320 uL Wash Buffer 1 to a final volume of 520 uL.

17 Add 520uL of Gradient medium to sample (Vf = 1040 uL).

Isolation by centrifugation

18 Layer 770 uL of 29% Cushion in the ultracentrifuge tube.

19 Layer the sample on top of cushion using a P1000, without disturbing the cushion.

20 Optional: Check tube weight, adjust weight to counter differences.

21 Centrifuge at least 3,000 rcf in a swinging bucket, at 4°C for 20 minutes with brake off.

22 Remove supernatant, remove the lower supernatant with P200, leaving about 50-100 uL.

Resuspension and permeabilisation

23 Gently resuspend nuclei in ultracentrifuge tube and transfer to 1.5 mL DNA lobind tube.

24 Rinse ultracentrifuge tube with Wash buffer 2 and transfer also to 1.5 mL DNA lobind tube.

25 Centrifuge @ 350-450xg for 5 mins. Discard the supernatant.

26 Resuspend in 0.1x lysis buffer (200 uL) and gently pipetmix 5x

27 Incubate on ice for 2 min

28 Add Wash buffer 2 (1mL) & gently pipetmix 5x

29 Centrifuge @ 350-450xg for 5 mins. Discard the supernatant.

30 Resuspend in 1X Diluted Nuclei buffer. Count