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Nuclei isolation from brain tissue for single-cell ATAC

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We use this protocol and it's working

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Abstract

This protocol is for isolating nuclei from brain tissue and prepare them for single-cell ATAC assays like the 10X Genomics Chromium Next GEM Single Cell ATAC Kit, HyDrop-ATAC, etc.

Attachments

XLSX

Nuclei_Isolation_ATA...

18KB

Materials

	A	B	C	D	E	F
	2X HB- (Homogenization Buffer)	**Stock conc**		**Final conc**		**V (µl)**
	Sucrose	2000	mM	640	mM	2245,6
	NaCl	5000	mM	20	mM	28,1
	MgCl2	1000	mM	6	mM	42,1
	Tris pH7,5	1000	mM	20	mM	140,4
	EDTA	500	mM	0,2	mM	2,8
	BSA	7,5	%	0,08	%	74,9
	Tween20	10	%	0,02	%	14,0
	Protienase inhibitor	50	X	2	X	280,7
	DTT	1000	mM	2	mM	14,0
	Water					4175,0
	Total Volume					**7017,6**

	A	B	C	D	E	F
	1X HB+ (Homogenization Buffer)	**Stock conc**		**Final conc**		**V (µl)**
	2X HB-	2	X	1	X	2200,0
	Igepal	10	%	0,05	%	22,0
	Digitonin	2	%	0,0025	%	5,5
	Water					2178,0
	Total Volume					**4400,0**

	A	B	C	D	E	F
	1X HB- (Homogenization Buffer)	**Stock conc**		**Final conc**		**V (µl)**
	2X HB- (Homogenization Buffer)	2	X	1	X	3648,0
	Water					3648,0
	Total Volume					**7296,0**

	A	B	C	D	E	F
	GM (Gradient Medium)	**Stock conc**		**Final conc**		**V(µl)**
	CaCl2	1000	mM	5	mM	12,5
	Optiprep	60	%	50	%	2080,0
	MgCl2	1000	mM	3	mM	7,5
	Tris pH 7.5	1000	mM	10	mM	25,0
	BSA	7,5	%	0,04	%	13,3
	Protienase inhibitor	50	X	0,2	X	10,0
	DTT	1000	mM	1	mM	2,5
	Water					345,3
	Total Volume					**2496,0**

	A	B	C	D	E	F
	ODM (Optiprep Diluent Medium)	**Stock conc**		**Final conc**		**V(µl)**
	KCl	3000	mM	150	mM	114,6

	A	B	C	D	E	F
	MgCl2	1000	mM	30	mM	68,7
	Tris pH 8	1000	mM	60	mM	137,5
	Sucrose	2000	mM	250	mM	286,4
	Water					1684,3
	Total Volume					**2291,5**

	A	B	C	D	E	F
	29% Cushion	**Stock conc**		**Final conc**		**V(µl)**
	Optiprep	60	%	29	%	1786,4
	ODM					1909,6
	Total Volume					**3696,0**

	A	B	C	D	E	F
	Resuspension Buffer	**Stock conc**		**Final conc**		**V(µl)**
	20X Nuclei buffer	20	X	1	X	22
	BSA	7,5	%	1	%	58,7
	Water					359,3
	Total Volume					**440**

A. Homogenization

1 Cut brain piece on dry ice, then transfer immediately in homogenizer containing 1 mL of ice-cold HB+. If tissue is still frozen, leave it in HB for 3 min before starting homogenization to prevent the nuclei from breaking.

2 Homogenize tissue with 10 manual gentle strokes (pestle A) + 10 manual gentle strokes (pestle B).

3 Put homogenate through 70 µm cell strainer over a 50 mL falcon (or immediately a 2 mL protein lo-bind tube).

4 Wash homogenizer & strainer with 1 mL HB- to a final volume of 2 mL.

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

6 Spin nuclei @500g for 5 min in a swinging bucket and remove supernatant.

7 Resuspend pellet with HB- to get a total volume of 520 µL and transfer to a clean DNA lo-bind tube.

8 Add 520 µL of Gradient medium (GM) to sample (Vf = 1040 µL).

B. Isolation by centrifugation

9 Layer 770 µL of 29% Cushion in a 2 mL DNA LoBind tube.

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

11 Centrifuge at least 3,000 rcf (you can go as high as 9,000) in a swinging bucket, at 4°C for 20 minutes with brake off.

12 Remove supernatant, remove the lower supernatant with P200, leaving about max 30 µL.

C. Resuspension and counting

13 Disrupt nuclei on bottom by pipetting with P200. And bring X µL to a fresh 1.5 mL LoBind tube.

14 Add X µL of 1X Resuspension buffer to tube to retain extra nuclei, and add them to the 1.5 mL tube from C.1.

15 Spin 5 min @ 350-450xg followed by resuspension in 1X Diluted nuclei buffer. Optional filter and count.