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Sample preparation for single nuclei sequencing of brain

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Human Protein Atlas



ABSTRACT

Sample preparation for single nuclei sequencing using fresh frozen tissue from human postmortem brain. The protocol is a combination of an already existing and published protocol in Nature protocols *Using single nuclei for RNA-seq to capture the transcriptome of postmortem neurons* and the 10x Genomics handbook *Sample preparation, isolation of nuclei for single nuclei RNA sequencing*.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Krishnaswami SR, Grindberg RV, Novotny M, Venepally P, Lacar B, Bhutani K, Linker SB, Pham S, Erwin JA, Miller JA, Hodge R, McCarthy JK, Kelder M, McCorrison J, Aevermann BD, Fuertes FD, Scheuermann RH, Lee J, Lein ES, Schork N, McConnell MJ, Gage FH, Lasken RS. Using single nuclei for RNA-seq to capture the transcriptome of postmortem neurons. Nat Protoc. 2016 Mar;11(3):499-524. doi: 10.1038/nprot.2016.015. Epub 2016 Feb 18. https://www.ncbi.nlm.nih.gov/pubmed/26890679

PROTOCOL STATUS

Working

We use this protocol in our group and it worked.

MATERIALS

NAME V	CATALOG # ~	VENDOR ~
Optiprep (lodixanol)	D1556-250ML	Sigma Aldrich
Sucrose	\$0389	Sigma Aldrich
RNaseZap® RNAse Decontamination Solution	AM9780	Life Technologies
Triton X-100	T8787	Sigma Aldrich
Protector RNase Inhibitor	3335402001	Sigma – Aldrich
LS Columns	130-042-401	Miltenyi Biotec
Buffer Kit RNase-free	AM9010	Thermo Fisher Scientific
Nuclease-Free Water	AM9939	Thermo Fisher Scientific
Dithiothreitol (DTT)	707265ML	Thermo Fisher Scientific
cOmplete™ EDTA-free Protease Inhibitor Cocktail	11873580001	Sigma Aldrich
Myelin Removal Beads II human mouse rat	130-096-733	Miltenyi Biotec
Ultra pure BSA (50 mg/mL)	cat# AM2616	Thermo Fisher Scientific

NAME V	CATALOG #	VENDOR V
SUPERase• In™ RNase Inhibitor (20 U/μL)	cat# AM2694	Thermo Fisher Scientific
PBS 1x without calcium & magnesium	Cat# 21-040-CVR	VWR International

MATERIALS TEXT

Buffers:

Nuclei isolation medium 1 (NIM1)

Component	Volume (µI)	Final concentration (mM)
1.5 M sucrose	2500	250
1 M KCl	375	25
1 M MgCl2	75	5
1 M Tris buffer	150	10
Nuclease-free water	11900	-
Total volume	15000	-

Important! This buffer should be prepared in advance, it can be stored in 50-ml tube at 4C for up to 6 months.

Nuclei isolation medium 2 (NIM2)

Component	Volume (µl)	Final concentration (mM)
NIM1	4895	
1 mM DTT	5	1 μΜ
50x protease inhibitor	100	1x
Total volume	5000	

Important! This buffer should be prepared fresh and placed at 4C or on ice for use. Afterwards, it should be discarded

Homogenization buffer

Component	Volume (µI)	Final concentration (mM)
NIM2	1455	1 x
RNaseIn 40 U μl-1	15	0.4 U μl-1
Superasin 20 U μl-1	15	0.2 U µl-1
Triton X-100 10% (v/v)	15	0.1% (v/v)
Total volume	1500	

Important! This buffer should be prepared fresh, protected from the light and placed at 4C or on ice for use. Afterwards, it should be discarded.

Nuclei Wash and Resuspension Buffer

1x PBS

1% BSA

0.2 U ul-1 Rnase inhibitor

LS Column Calibration Buffer

1x PBS

0.5% BSA

Iodixanol medium (IDM)

Component	1× volume (μl)	Final concentration (mM)
1.5 M sucrose	2500	250

1 M KCl	2250	150
1 M MgCl2	450	30
1 M Tris buffer, pH 8.0	900	60
Nuclease-free water	8900	-
Total volume	15000	

The medium can be stored at 4C for up to 6 months.

lodixanol dilutions

Component	1× volume (μl)	Final concentration
Iodixanol 60% (vol/vol)	12500	50% vol/vol
IDM	2500	-
Total volume	15000	
Component	1× volume (μl)	Final concentration
Iodixanol 60%	7250	29% vol/vol
IDM	7750	-
Total volume	15000	

The reagents can be stored at 4C for up to 6 months.

SAFETY WARNINGS

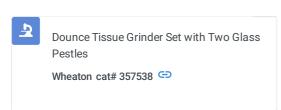
Regulations about working with tissue samples may vary between institutions, it is important to be aware about the guidelines before to start any experiment.

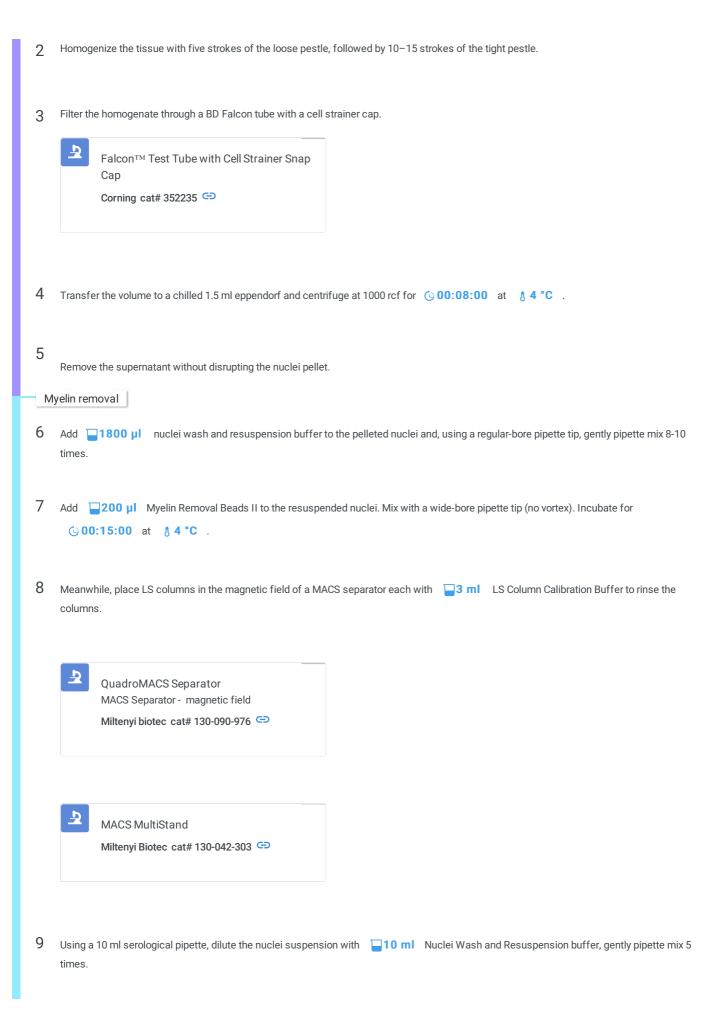
BEFORE STARTING

- Run Bioanalyser to check RNA integrity number (RIN) and evaluate the quality of the tissue.
- Clean workstation, pipettes and tools with RNaseZap solution before to start the experiment.
- Precool the Dounce homogenizer and pestles on ice. Once they are cooled, fill the homogenizer with 1ml of cold homogenization buffer and keep it on ice.

Nuclei isolation

1 Place a Petri dish on dry ice and transfer the tissue there, cut it using a chilled scalpel. Transfer the tissue into the precooled Dounce homogenizer and keep the homogenizer on ice during the rest of the protocol (to reduce heat caused by friction).





10 Centrifuge the nuclei at 500 rcf for (00:10:00 at 4 °C . 11 Remove the supernatant without disrupting the nuclei pellet and add 22 ml Nuclei Wash and Resuspension buffer. 12 Apply 1 ml nuclei suspension in each column. 13 Wash each of the columns with 11 ml Nuclei Wash and Resuspension buffer 5 go to step #13 once more. Collect the effluent into two 5ml Eppendorf tubes. 14 15 Centrifuge the two vials with nuclei at 500 rcf for (00:05:00 at 4 °C . 16 Remove the supernatant from the two vials without disrupting the nuclei pellet and add 🔲 500 µl Nuclei Wash and Resuspension buffer. 17 Pool both resuspended nuclei in a new pre-chilled 1.5 ml Eppendorf. Density gradient centrifugation 18 Centrifuge at 1000 rcf for **© 00:08:00** at **§ 4 °C**. Meanwhile, prepare the iodixanol dilution mix (IDM) and iodixanol dilutions to place them on ice. 19 Aspirate the supernatant (\sim 1,000 μ I) and gently resuspend the pellet in 250 μ I of homogenization buffer. 20 Strain the mixture through a BD Falcon tube with a cell strainer cap to remove any remaining aggregates, and place it on ice. 21 Gently mix the nuclei with 250 µl of 50% (vol/vol) iodixanol, and, to a new Eppendorf tube, add 500 µl of 29% iodixanol. Slowly layer 500 µl of 22 the nuclei mixture over the 29% iodixanol. 23 Centrifuge at 13.500 rcf for (00:20:00 at 4 °C .

- Remove and discard the supernatant without disrupting the nuclei pellet.
- 25 Resuspend the final pellet in P60 µl Nuclei Wash and Resuspension buffer. The sample is ready for QC-nuclei counting.

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