Jan 08, 2020

Nuclei Isolation for SNARE-seq2

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1 Works for me dx.doi.org/10.17504/protocols.io.8tvhwn6

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ABSTRACT

This protocol is intended to be used for the isolation of nuclei from fresh-frozen brain tissue in preparation for analysis by Single-Nucleus Chromatin Accessibility and mRNA Expression sequencing (SNARE-seq). It has been applied to tissues from mouse, marmoset, and human.

GUIDELINES

This protocol is designed specifically for isolating tissue for SNARE-seq. RNA stability is considered at every step. Samples are kept on ice throughout the process, all centrifuges are pre-chilled to 4 ^{o}C before use, and RNase Away is used to spray down all surfaces and pipets before use.

MATERIALS

NAME	CATALOG #	VENDOR
Sucrose	S3-212	Fisher Scientific
Protease Inhibitor Tablets cOmplete Mini EDTA free	11836170001	Roche
RNase Inhibitor	2313A	Takara
Bovine Serum Albumin	700-107P	Gemini Bio-Products
Magnesium acetate tetrahydrate	M5661	Sigma-aldrich
Calcium chloride dihydrate	C5080	Sigma Aldrich
Ethylenediaminetetraacetic acid (EDTA)	EDS	Sigma Aldrich
Triton X-100	T8787	Sigma Aldrich
16% Formaldehyde (w/v) Methanol-free	28906	Thermo Fisher Scientific
DAPI	10236276001	Sigma Aldrich
50um filters	04-0042-2317	Sysmex
Tissue Homogenizer	358005	

Buffer Preparation

1 **Nuclei Extraction Buffer (NEB):**

	Final Concentration		Chemical to Add	Stock Concentration
Sucrose	320 mM		5.48g	Solid
Magnesium Acetate	3 mM		32.16mg	Solid
CaCl2	5 mM		50µl	5M Stock
EDTA	100 µM		10µl	0.5M Stock
Tris-HCl pH 8	10 mM		500µl	1M Stock
Triton X-100	0.1 %		500µl	10% Stock
MilliQ Water			Bring to 50ml	
RNase Inhibitor	80 U/ml		100µl	40,000U/ml
Protease Inhibitor Tablet			1 Tablet	

Prepare NEB as outlined above. 50ml is enough for 3 samples. Combine reagents 2-8 the night before nuclei isolation and chill at 4 ^{O}C. Add RNase inhibitor and Protease Inhibitor tablet the morning of isolation.

2 **PBSE + Sucrose:**

	Final Concentration		Chemical to Add	Stock Concentration
Sucrose	320 mM		5.48g	Solid
EGTA	250 µM		25µl	0.5M
1x PBS pH 7.4			Bring to 50ml	
RNase Inhibitor	40 U/ml		50µl	40,000U/ml
Protease Inhibitor Tablet			1 Tablet	

Prepare PBSE + Sucrose as outlined above. 50ml is enough for 3 samples. Combine reagents 2-4 the night before nuclei isolation and chill at 4 ^{O}C. Add RNase inhibitor and Protease inhibitor tablet the morning of isolation.

3 **PBSE + BSA:**

	Final Concentration		Chemical to Add	Stock Concentration
BSA	1%		500mg	Solid
EGTA	250 µM		25µl	0.5M
1x PBS pH 7.4			Bring to 50ml	
RNase Inhibitor	40 U/ml		50µl	40,000U/ml
Protease Inhibitor Tablet			1 Tablet	

Prepare PBSE + BSA as outlined above. 50ml is enough for 3 samples. Combine reagents 2-4 the night before nuclei isolation and chill at 4 ^{O}C. Add RNase inhibitor and Protease inhibitor tablet the morning of isolation.

Tissue Dissociation

4 Remove tissue from -80^{O}C storage and place on ice.

5 Image the tissue rapidly in front of a ruler

6 Add 1ml of ice cold NEB to the tissue and incubate on ice for **00:15:00**

7 During the incubation, wash the homogenizer with MilliQ water, 10% bleach, 70% EtOH, and MilliQ water again

8 Rinse homogenizer with 1ml of ice cold NEB

9 Once the incubation is complete, add 1 ml of fresh NEB to the homogenizer and transfer the tissue and the 1ml of NEB it is in to the homogenizer

10 Homogenize the tissue using ~20 compressions with the pestle, or until the tissue is entirely dissociated, this step can be variable

11 Pass nuclei suspension through 50 micron filter into a 15 ml conical tube, wash filter with another 4ml of NEB

12 Wait for **00:05:00**

13 Centrifuge the samples **820 x g , 5 min**

14 Carefully aspirate the supernatant and slowly resuspend the pellet in 1ml of NEB

15 Gently add another 9 ml of NEB to the sample

16 Centrifuge the samples **820 x g , 5 min**

Nuclei Fixation

17 Aspirate the supernatant and slowly resuspend the samples in 1ml of PBSE + Sucrose

18 Gently add another 5 ml of PBSE + Sucrose

19 Centrifuge the samples **820 x g , 5 min**

20 Aspirate all but approximately **100 µl** of the PBSE+Sucrose, and gently resuspend the nuclei pellet in the remaining volume

21 While gently vortexing, add 5ml of 0.5% Formaldehyde diluted in 1X PBS

22 Fix for 10 min on ice

23 Centrifuge the samples **820 x g , 5 min**

24 Aspirate the supernatant and slowly resuspend the samples in 1ml of PBSE + Sucrose

25 Centrifuge the samples **820 x g , 5 min**

DAPI Staining and Sorting

26 Aspirate the supernatant and slowly resuspend the samples in 1ml of PBSE + BSA

27 Gently add another 10ml of PBSE + BSA

28 Centrifuge the samples **820 x g , 5 min**

29 Make 1ml/sample of PBSE + BSA + DAPI by adding DAPI at a final concentration of 1.25 µg/ml to PBSE + BSA. This is a 1:4000 dilution from a DAPI stock at 5mg/ml

30 Aspirate the supernatant and slowly resuspend the samples in 1ml of PBSE + BSA + DAPI

31 Pass nuclei suspension through 50 micron filter into a FACS tube, gently tap on table to get all nuclei through the filter

32 Use the DAPI singlet peak to sort singlet nuclei events into a 2ml low binding eppendorf tube.

33 Store all samples on ice until SNARE-seq processing begins. Do not store for longer 4 hours before processing.