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 We use this protocol and it's working

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Nuclei Isolation for SNARE-seq2

Carter Palmer¹, Christine Liu¹, Jerold Chun²

¹University of California, San Diego, and Sanford Burnham Prebys Medical Discovery Institute;

²Sanford Burnham Prebys Medical Discovery Institute

BICCN

Carter Palmer

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 °C before use, and RNase Away is used to spray down all surfaces and pipets before use.

MATERIALS

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☒ Sucrose **Fisher Scientific Catalog #S3-212**

☒ Protease Inhibitor Tablets cOmplete Mini EDTA free **Roche Catalog #11836170001**

☒ RNase Inhibitor **Takara Catalog #2313A**

☒ Bovine Serum Albumin **Gemini Bio-Products Catalog #700-107P**

☒ Magnesium acetate tetrahydrate **Sigma-aldrich Catalog #M5661**

☒ Calcium chloride dihydrate **Sigma Aldrich Catalog #C5080**

☒ Ethylenediaminetetraacetic acid (EDTA) **Sigma Aldrich Catalog #EDS**

☒ Triton X-100 **Sigma Aldrich Catalog #T8787**

☒ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific Catalog #28906**

☒ DAPI **Sigma Aldrich Catalog #10236276001**

☒ 50um filters **Sysmex Catalog #04-0042-2317**

☒ Tissue Homogenizer **Contributed by users Catalog #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
CaCl ₂	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 °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 °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 °C. Add RNase inhibitor and Protease inhibitor tablet the morning of isolation.

Tissue Dissociation

- 4 Remove tissue from -80°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.