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

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):

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

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:**

<table>
<thead>
<tr>
<th>Chemical to Add</th>
<th>Stock Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.48g Solid</td>
<td></td>
</tr>
<tr>
<td>0.5M</td>
<td></td>
</tr>
<tr>
<td>40,000U/ml</td>
<td></td>
</tr>
<tr>
<td>1 Tablet</td>
<td></td>
</tr>
</tbody>
</table>

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:**

<table>
<thead>
<tr>
<th>Chemical to Add</th>
<th>Stock Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>500mg Solid</td>
<td></td>
</tr>
<tr>
<td>0.5M</td>
<td></td>
</tr>
<tr>
<td>40,000U/ml</td>
<td></td>
</tr>
</tbody>
</table>

protocol.io | https://dx.doi.org/10.17504/protocols.io.8tvhwn6 | Oct 8 2020
| 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 $820 \times g, 5$ 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 $820 \times g, 5$ 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 \( 820 \times g, 5 \text{ min} \)

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 \( 820 \times g, 5 \text{ min} \)

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

25 Centrifuge the samples \( 820 \times g, 5 \text{ min} \)
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 \(820 \times g, 5 \text{ min}\)

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.