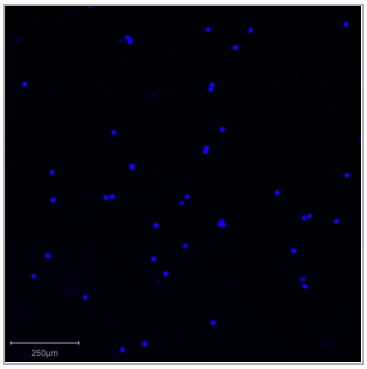

MAY 15, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.261genq1og47/v1

Protocol Citation: Luise Seeker, Sarah Jäkel, Anna Williams 2023. Nuclei isolation from human CNS samples using NUC201 isolation kit from Sigma-Aldrich for 10X single nuclei RNA sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261genq1og47/v1

MANUSCRIPT CITATION: https://doi.org/10.1101/2022.03.22.485367

Protocol status: Working
We use this protocol and it's working

Created: Aug 20, 2022

Last Modified: May 15, 2023

Nuclei isolation from human CNS samples using NUC201 isolation kit from Sigma-Aldrich for 10X single nuclei RNA sequencing

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ABSTRACT

Because retrieval of an unbiased group of viable cells from post-mortem human CNS is very challenging if not impossible to achieve, single nucleus RNA sequencing is often used as a very valuable alternative. However, nuclei isolation protocols deliver variable yields and nuclei quality. Here we share a reliable protocol using the Nuc201 kit by Sigma-Aldrich (NUC201-1KT) that delivers good quality nuclei for downstream single nuclear RNAseq profiling.

MATERIALS

We use additionally to the reagents provided with the kit:

- Starlab low binding microcentrifuge tubes: SLE1415-2600 - (STARLAB UK)
- Starlab filter pipette tips: SLS1120-8810 - (STARLAB UK) & SLS1182-1830 - (STARLAB UK)
- RNAse Inhibitors: AM2694 - (Thermo Fisher Scientific (Life Technologies)))
- Trypan Blue: 1450013 - (Bio-Rad Laboratories)
- Counting slides: 1450011 - (Bio-Rad Laboratories)
- DTT 0.1 M (protocol recommends to buy powder and to make up 1M solution. We used 0.1M which we had in solution in our freezer
- Razor blades: Solimo 100 double edge blades
- Syringes: Terumo SS+01T1
- Needles: BD microlance, Gauge - 27G, Length - 0.5" (13mm), External Diameter - 0.4mm, sterile, SKU: 01232
- Insulin needles: VWR, BDAM324892, 0,5ML 29G 12,7MM NEEDLE
- cell strainer: Sysmex / Partec CellTrics 30µm filter
- Strip tubes: Eppendorf PCR Tubes, 0.2 mL, PCR clean, 8-tube strips, 120 pcs. (960 tubes) Catalog No. 0030124359

PROTOCOL integer ID:
68954

Keywords: nuclei, 10X Genomics, human CNS, brain, nuclei isolation, fresh-frozen

BEFORE START INSTRUCTIONS

For adult human brain samples we usually use cryosectioned tissue (20 microm). For more delicate tissues such as the brains of foetuses, it might be a better approach to cryo-section them directly into Eppendorf tubes, as the treatment will be more gentle this way. For those delicate samples we also avoid the use of needles and homogenise them simply by adding lysis buffer directly to the tubes and by inverting them gently several times.

1 Prepare Lysis Buffer (200ul per sample): for 8 samples: 1700 ul PURE buffer, 17ul 0.1M DTT, 17 ul 10% Triton, 34ul RNAseinhibitor

2 Prepare sucrose Cushion: (560 ul per sample): for 8 samples: 5ml PURE 2M Sucrose solution, 550ul Sucrose Cushion buffer, 55ul 0.1M DTT, 100ul RNAseInhibitor

3 Retrieve cryosectioned tissue (we use 20 micrometre thickness) from freezer and place in Laminar flow hood. Space slides out so that they can thaw and dry. This will take ~ 20 min.

4 Prepare three 1.5 ml low binding microcentrifuge tubes (see materials section) per sample and label them.

5 Use razor blades (see materials section) to scrape off the (desired piece of) tissue from the slides and place the tissue in the first corresponding microcentrifuge tube.

6 Add 200 ul of the prepared lysis buffer (prepared in step 1) to each tube and homogenise the tissue with needles (descending needle size:see materials section). Avoid making bubbles.

7 Mix each 200ul lysate with 360ul Sucrose Cushion (prepared in step 2) and keep on ice

8 Filter through a 30um filter (see materials section) into the second prepared microcentrifuge tube. If sample does not flow through within a few minutes, aspiration with a pipette and use of a second filter may be required.

9 Add 200ul of Sucrose Cushion to the third microcentrifuge tube (prepared for each sample in 2) and carefully overlay with 560ul filtrate from step 8.

10 Centrifuge for 45min at full speed (16,100 g, 4 degrees Celsius).

11 Prepare elution solution: For 8 samples mix 7 ml of PURE storage buffer with 0.2U/ul RNASE-Inhibitor (70ul) and keep on ice.

12 After centrifugation, a small pellet should be visible. Remove the supernatant.

13 Re-suspend pellet in 100 ul elution solution (from step 11) and transfer into fresh tube. This avoids myelin that is stuck to the inside of the microcentrifuge tube to be washed down during the next centrifugation step where it will impact the purity of the isolated nuclei. After transferring the sample to the new tube, add another 100 ul of elution solution to each sample.

14 Centrifuge for for 5 min at 1000g.

15 There should be a visible pellet. If not, check if centrifuge settings were correct before proceeding.

16 Re-suspend in 200 ul elution solution.

17 Centrifuge for for 5 min at 1000g

18 There should be a visible pellet in some of the samples. If not, check if centrifuge settings were correct before proceeding.

19 Remove supernatant but keep supernatant and measure free DNA using a NanoDrop. This may give a rough indication of how much free RNA may be in the sample. This did help us to trouble shoot nuclei extractions of more fragile samples such as foetus samples.

20 Re-suspend pellet in 50 -150 ul elution solution.

21 Count nuclei using the BioRad cell counter (Biorad TC200) using Trypan blue and counting slides (see materials section). Mix 5 ul of Trypan blue with 5 ul of sample and transfer the whole 10 ul onto the counting slide. If images are required, use USB stick to save them).

22 Standardise nuclei concentration to 100,000 nuclei per ml using the elution solution and follow 10X genomics protocol for snRNAseq.