Nov 27, 2024

Nuclei Isolation and Clean-Up using GentleMACS and Anti-Nucleus Microbeads

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4w2k2vo5/v1

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Protocol Citation: Fabian Köchl 2024. Nuclei Isolation and Clean-Up using GentleMACS and Anti-Nucleus Microbeads. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4w2k2vo5/v1

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

Created: October 30, 2024

Last Modified: November 27, 2024

Protocol Integer ID: 111241

Keywords: clean nuclei suspension, liver nuclei suspension, nuclei rna, nuclei isolation, alternative nuclei, nuclei quality, prone to high ambient rna content, high ambient rna content, gentlemacs octo dissociator, using gentlemac, nuclei, rna, activated cell sorting

Abstract

Obtaining a clean nuclei suspension for subsequent single-nuclei RNA sequencing can be challenging. Especially liver nuclei suspensions are prone to high ambient RNA content.
Alternative nuclei clean up methods like Fluorescence-activated Cell Sorting can stress nuclei, decrease nuclei quality and are time consuming and need specialized equipment.

To overcome these challenges we applied a simple protocol for isolating and cleaning up nuclei for single-nuclei RNA sequencing using the GentleMACS Octo dissociator coupled with anti-Nucleus microbeads cleanup.
The following protocol has been successfully tested for Rat, Mouse and Minipig liver as well as Mouse brain.

Attachments

PDF
NucleiExtractionBuff...
89KB

PDF
Anti-Nucleus Mircobe...
189KB

Materials

gentleMACS Octo Dissociator with Heaters (Miltenyi, # 130-096-427)
gentleMACS Octo Coolers (Miltenyi,#130-130-533)
QuadroMACS Separator (Miltenyi,#130-090-976)
MACS MultiStand (Miltenyi, # 130-042-303)
Refrigerated Centrifuge

Anti-Nucleus MicroBeads (Miltenyi,# 130-132-997)
Nuclei Extraction Buffer (Mliltenyi# 130-128-024)
MACS BSA Stock Solution (# 130-091-376) or BSa 30% to be checked
MACS SmartStrainers (70 µm) (# 130-098-462)
MACS SmartStrainers (30 µm) (# 130-098-458) or CellTrics (Sysmex, #04-004-2326)
gentleMACS C Tubes (# 130-093-237, # 130-096-334)
LS Columns (# 130-042-401)
RNase inhibitor (e.g. RiboLock RNase Inhibitor)
Phosphate-buffered saline (PBS), pH 7.2

1. Preparations

1 Pre-cool the buffers, and consumables with sample contact (e.g. gentleMACS C Tube and SmartStrainers) at 4 °C Overnight Place the gentleMACS Octo Coolers in -20 °C Overnight

2 Pre-cool the centrifuge to 4 °C before the start of the experiments

3 Per extraction, add RNase inhibitor (final concentration [M] 0.2 U/µL) to pre-cooled 4 mL Nuclei Extraction Buffer.

4 Prepare Nuclei separation buffer (for resuspension) before further enrichment of the nuclei using Anti-Nucleus MicroBeads.

Per sample:

1x PBS: 10 ml
Nuclei Extraction Buffer: 1667 µL
BSA (30%): 15.6 µL
RNAse inhibitor (0.2U/ul): 58.3 µL

> Note
>
> Degas buffer before use, as air bubbles could block the column.

5 In a pre-cooled Petri dish on dry ice, cut a fresh frozen tissue sample into a piece of 25-50 mg with a pre-cooled scalpel.

> Note
>
> Make sure that the sample is always on dry ice.

Nuclei Extraction

6 Add 2 mL ice-cold lysis buffer to each pre-cooled gentleMACS C Tube.

7 Transfer tissue pieces to the gentleMACS C Tube containing lysis buffer

8 Close gentleMACS C Tube and place it on the gentleMACS Dissociator.

9 Retrieve gentleMACS Octo Coolers form the freezer and attach them over the C tubes on the GentleMACs

10 Run gentleMACS Program **4C_nuclei_1** on the gentleMACS Dissociator. Detach the tube after the program finished and place it on ice

11 Apply nuclei suspension to a MACS SmartStrainer (70 µm) (from brain tissue samples : 100uM) placed on
a 5 mL tube

12 Wash MACS SmartStrainer with 2 mL ice-cold lysis buffer

13 Discard MACS SmartStrainer and centrifuge nuclei suspension at 5m
30 x g, 4°C, 00:05:00 .
Carefully aspirate supernatant completely.

14 Resuspend nuclei pellet with 1.5 ml ice-cold resuspension buffer by slowly and gently pipetting the sample up and down for 10 times

15 Apply nuclei suspension to a Celltrics Filter (30 µm)on a 1.5 mL tube or a MACS SmartStrainer (30µm) placed on a 5 mL tube.

16 Determine the nucleus number preferably using an automated Cell Counter

3 Magnetic Labelling 5m

17 Transfer the volume that corresponds to 2 million nuclei to a new 1.5ml Eppi and centrifuge 300 x g, 4°C, 00:05:00 . Carefully remove supernatant 5m

18 Resuspend nucleus pellet in 900 µL of nuclei separation buffer and transfer to 5ml tube

19 Add 100 µL of Anti-Nucleus MicroBeads

20 Mix well and incubate for 15 min 4 °C

21 Add 2 mL nuclei separation buffer and proceed to magnetic separation

Magnetic separation

22 Place LS column in the magnetic field of a suitable MACS Separator

23 Prepare column by rinsing with 3 mL of nuclei separation buffer

24 Apply nucleus suspension onto the column. Collect flowthrough containing debris

25 Wash column two times with 1 mL of nuclei separation buffer. Collect debris that passes through and combine with the flow-through from previous step

26 Remove column from the separator and place it on a 1.5ml Eppi

27 Pipette 1 mL nuclei separation buffer onto the column. Immediately flush out the magnetically labeled nuclei by firmly pushing the plunger into the column.

QC and preparation for Single Nucleus RNA sequencing 5m

28 Centrifuge 300 x g, 4°C, 00:05:00 , remove supernatant 5m

29 Resuspend the pellet in 500 µL of PBS + 0.04% BSA (with RNAse inhibitor at 0.2U/ul).

30 Determine the nuclei concentration preferably using an Fluorescence-based automated Cell Counter

31 Dilute the sample with PBS + 0.04% BSA to the recommended concentration for single nuclei RNA sequencing