OCT 03, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gp3bo8vzp/v1

Protocol Citation: Deepak Balamurali, Kinga M. Wrona, Lea Eich, Anika Witten, Alba M. Albert, Marc N. Hirt, Thomas Eschenhagen, Monika Stoll 2023. GONE in 360 seconds: gentleMACS Octo Dissociator-based Nuclei Extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp3bo8vzp/v1

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

GONE in 360 seconds: gentleMACS Octo Dissociator-based Nuclei Extraction

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ABSTRACT

Single-cell and single-nuclei approaches are being extensively used to decipher the cellular heterogeneity of samples and to understand the genetic factors leading to various diseases. While single-cell RNA sequencing is quite straightforward, single-nuclei sequencing requires careful consideration in the isolation & processing of intact nuclei after dissociation. Numerous protocols have been developed for this purpose, but the size and morphology of cardiomyocytes make the process quite complicated.[1-3]

Previously described processes also included steps involving manual homogenization using a Dounce homogenizer and Fluorescence-activated Cell Sorting.[2,4] These steps have an increased propensity to vary from user to user and also produce stress on the cells, which further alters the transcriptional profile of these nuclei.

To this end, we devised a simple direct protocol for processing cardiac tissue into intact nuclei for downstream single-nuclei RNA sequencing using the gentleMACS Octo Dissociator. This protocol has been extensively tested with Engineered Heart Tissues (EHTs) and also works well with frozen or fresh cardiac tissues from human biopsies.

Created: Jul 26, 2023

Last Modified: Oct 03, 2023

PROTOCOL integer ID: 85513

Keywords: Nuclei Extraction, Nuclei Isolation, Heart, Cardiomyocytes, gentleMACS

MATERIALS

Instruments:

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

Consumables & Reagents:

Nuclei Extraction Buffer (# 130-128-024)
MACS BSA Stock Solution (# 130-091-376)
MACS SmartStrainers (70 µm) (# 130-098-462)
MACS SmartStrainers (30 µm) (# 130-098-458)
gentleMACS C Tubes (# 130-093-237, # 130-096-334)
LS Columns (# 130-042-401)
RNase inhibitor (e.g. murine RNase Inhibitor from New England Biolabs)
Phosphate-buffered saline (PBS), pH 7.2

SAFETY WARNINGS

See SDS (Safety Data Sheet) for safety warnings and hazards.

BEFORE START INSTRUCTIONS

All samples and reagents are kept on ice or at 4 °C at all steps of the protocol.
Prepare all buffers and reagents as described in the "Pre-processing" section.

1. Pre-processing

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

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

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

4 Prepare the nuclei separation buffer (for resuspension) before further enrichment of the nuclei using Anti-Nucleus MicroBeads. Dilute as specified below:

MACS BSA Stock Solution 1:250 (0.04% final concentration) and
Nuclei Extraction Buffer 1:7 (14% final concentration) in phosphate-buffered saline (PBS),
pH 7.2

In case of RNAbased downstream applications, add RNase inhibitor (final concentration 0.2 U/µL).

> **Note**
>
> The datasheet from Miltenyi Biotec carries detailed information on the volume of resuspension buffer required based on the amount of tissue used as starting material.

5 Retrieve fresh/frozen tissue 50-100 mg and cut into small pieces using sterile forceps and a surgical blade.

> **Note**
>
> The tissue must be cut up preferably before freezing. Adequate care must be taken to ensure that the tissue does not thaw completely.

2. 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 Nuclei Extraction Buffer and directly

proceed with the following steps until samples are dissociated. Close the gentleMACS C Tube and place it on the gentleMACS Dissociator.

Note

Make sure that the tissue is floating freely in the buffer and not adhering to any parts of the tube or the cap.

8 Place previously cooled gentleMACS Octo Coolers on the C tubes to help maintain the temperature.

9 Run gentleMACS Program **4C_nuclei_1** on the gentleMACS Octo Dissociator.

10 After termination of the program, detach C Tube from the gentleMACS Dissociator and place the C Tube immediately on ice.

11 Apply nuclei suspension to a MACS SmartStrainer (70 µm) placed on a 15 mL tube. Wash MACS SmartStrainer with 2 mL ice-cold Nuclei Extraction Buffer.

12 Discard MACS SmartStrainer and centrifuge nuclei suspension at 300 x g, 4°C for 00:05:00. Carefully aspirate supernatant completely.

13 Resuspend nuclei pellet with ice-cold nuclei separation buffer slowly and gently pipetting the sample up and down 10 times.

14 Apply nuclei suspension to a MACS SmartStrainer (30 µm) placed on a 15 mL tube.

15 Collect nuclei suspension and proceed immediately with downstream applications (in this case, purification with Anti-Nucleus MicroBeads)

3. Magnetic Labelling & Purification

16 Determine the nucleus number using an automated Cell Counter or Neubauer chamber.

17 Centrifuge nucleus suspension at 300 x g, 4°C for 00:05:00. Pipette off supernatant completely.

> Note
>
> By resuspending in a relatively small volume at the top, the nuclei are more concentrated. This saves the need for this centrifuge step and avoids the risk of losing nuclei.

18 Resuspend 1x 10^6 nuclei from the pellet in 450 µL of nuclei separation buffer.

19 Add 50 µL of Anti-Nucleus MicroBeads to each reaction tube.

20 Mix well and incubate for 00:15:00 in the refrigerator (2-8 °C).

21 Add 2 mL nuclei separation buffer (independent from nucleus sample input) and proceed to magnetic separation.

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

> **Note**
>
> Both the QuadroMACS & MidiMACS separators can be used for this process. Please note that is important to use a compatible column as well.

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

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

25 Wash the column twice with 1 mL of nuclei separation buffer. Collect debris that passes through and combine with the flowthrough from the previous step.

26 Remove the column from the separator and place it on a suitable collection tube.

27 Pipette the appropriate amount of nuclei separation buffer onto the column depending on the nucleus sample input.

28 Immediately flush out the magnetically labeled nuclei by firmly pushing the plunger into the column. The nuclei extracted can directly be proceeded with for downstream applications such as single-nuclei RNA sequencing.

4. Quality Control (optional)

29 Add DAPI Staining Solution (final concentration of 0.25 µg/ mL) to a small fraction of the nuclei suspension. Mix by inverting gently.

30 Incubate for 00:05:00 at 4 °C.

31 Load sample on a hemocytometer or on a flow cytometer and analyze sample according to manufacturer's recommendation.