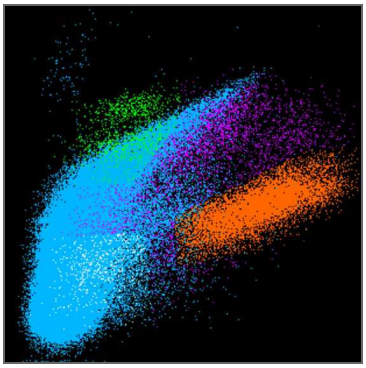

VERSION 2

JAN 26, 2024

OPEN ACCESS

Nuclei Isolation for HMBA FACS V.2

Lakme Caceres[1]

[1]Princeton Neuroscience Institute

L

Lakme Caceres
Princeton University

ABSTRACT

This protocol is for purifying nuclei for downstream 10X sequencing.

GUIDELINES

Keep tissue/nuclei on ice as much as possible.

DOI:
dx.doi.org/10.17504/protocols.io.kxygx35ywg8j/v2

Protocol Citation: Lakme Caceres 2024. Nuclei Isolation for HMBA FACS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx35ywg8j/v2Version created by Lakme Caceres

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

Created: Jan 18, 2024

Last Modified: Jan 26, 2024

PROTOCOL integer ID: 93752

Keywords: nuclei, isolation,
FACS

Prepare Stock Solutions

1 Make 50 mL **Nuclear Isolation Media** by filling a 100 mL bottle with 25 mL of MilliQ water and then adding 500 uL 1M Tris, 1.25 mL 1M KCl, 250 uL 1M MgCl2, and 4.28 g Sucrose. Shake until sucrose is dissolved then fill to 50 mL with MilliQ water. Filter it using a stericup into another clean bottle. (4°C - 2 weeks)

2 Make 20 mL **10% Triton X-100** by combining 18 mL MilliQ water with 2 mL Triton X-100 in a 50 mL tube. Vortex and then incubate at room temperature for 20 minutes. Filter it through a 0.22 µm filter with a syringe into a clean 50 mL tube. (4°C - 1 month)

For a smaller stock, make 1 mL by combining 900 uL MilliQ water with 100 uL Triton-X 100. Filter with a FlowMi after vortex and incubation.

3 Make 1 mL of **0.1 mg/mL DAPI** by adding 100 uL of 1 mg/mL DAPI stock to 900 uL of milliQ water. (4C - 1 month)

Prepare Fresh Solutions

4 Make 3 mL **Homogenization Buffer** by adding 2.892 mL Nuclear Isolation Media to a 5 mL eppendorf. Then add 60 uL protease inhibitor, 30 µL 10% Triton X-100, 15 uL RNase inhibitor, and 3 µL 100 mM DTT.

5 Make 15 mL **Blocking Buffer** by adding 13.425 mL 1X PBS, 1.5 mL 10% BSA, and 75 uL RNase inhibitor (0.2 U/uL) to a 15 mL falcon tube.

Homogenization

6 Clean dounce, scalpel, and forceps using MilliQ water, ethanol, RNase Zap, then MilliQ again. The red-tape forceps are for unfixed tissue.

7 Place your sample on dry ice. Tare a weigh boat or tube on the scale and weigh your sample.

8 Add tissue to dounce and push it to the bottom using 1 mL of Homegenization Buffer and the pestle. Homogenize the tissue without creating bubbles. Then add the remaining 2 mL of the Homogenization Buffer and continue to dounce until homogenized.

9 Pass all of the nuclei suspension through a 40 um filter.

10 Centrifuge at 900 g/rcf for 10 minutes at 4°C.

Blocking Buffer & Antibodies

11 Add 950 µL Blocking Buffer to each of your control tubes.

12 The amount of Blocking Buffer we will need to resuspend our nuclei pellet with after centrifuging depends on the number of controls we will run. Each control tube will require 50 µL of nuclei suspension, and our sample needs to have 1,000 µL of nuclei suspension left over. In this case we are running two controls.

13 After the cell homogenate is done centrifuging, discard the supernatant and resuspend the pellet in 1,100 µL of Blocking Buffer. Incubate for 15 minutes on ice.

14 Add 50 µL of nuclei suspension to each control tubes.

15 Add the corresponding antibodies to the control tubes:
- 2 µL NeuN-PE (1/500) to the PE-only control
- 0.5 µL Olig2-Alexa 488 (1/2000) to the A488-only control

16 Add both 2 µL NeuN-PE and 0.5 µL Olig2-Alexa 488 to the sample tube.

17 Place all tubes in a rotator placed in a dark 4°C fridge and incubate for 30 minutes.

Wash

18 After incubation, spin down all of the tubes at 500 g for 5 minutes at 4°C.

19 Decant and resuspend in 999 uL blocking buffer. Consider adding an additional wash step here.

20 Add 1 uL of 0.1 mg/mL DAPI, a final concentration of 0.1 ug/mL, to each sample and control tube and invert to mix.

21 Add 300 uL Blocking Buffer to falcon FACS tubes and label them A488+, PE+, and Double Negative. We will sort into these.

22 Store tubes on ice until FACS. Bring DAPI stock and Blocking Buffer with you to FACS in case we have to dilute the sample to lower the event count.