

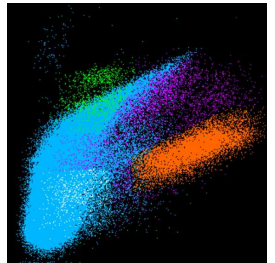
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Version 3

Nuclei Isolation for FACS V.3

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

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

Guidelines

Keep tissue/nuclei on ice as much as possible.

Materials

0.1 ug/uL Hoechst - 4C

Troubleshooting

Prepare Stock Solutions

- 1 Make 20 mL **10% BSA** by combining 2 mL of BSA with 18 mL of MilliQ water in a 50 mL falcon tube. (4°C - 2 weeks)
- 2 Make 250 mL **Nuclear Isolation Media** by filling a 250 mL bottle with 200 mL of MilliQ water and then adding 2.5 mL 1M Tris, 6.26 mL 1M KCl, 1.25 mL 1M MgCl₂, and 21.45 g Sucrose. Shake until sucrose is dissolved then fill to 250 mL with MilliQ water. (4°C - 2 weeks)
- 3 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)

Prepare Fresh Solutions

- 4 Make 3 mL **Homogenization Buffer** by adding 2.9 mL Nuclear Isolation Media (filtered via syringe) to a 5 mL eppendorf. Then add 3 µL 100 mM DTT and 30 µL 10% Triton X-100.
- 5 Make 10 mL **Blocking Buffer** by adding 8.2 mL MilliQ water, 1 mL 10X PBS, and 800 µL 10% BSA to a 15 mL falcon tube and vortex.

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 Get tissue sample from -80°C freezer and place on dry ice. Carve out tissue on an ice block then weigh out approximately 60 mg on a tared weigh boat.
- 8 Add tissue to dounce and push it to the bottom using 1 mL of Homogenization 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 three FlowMi filters, 1000 µL at a time into a new 5 mL eppendorf.
- 10 Centrifuge at 900 g/rcf for 10 minutes at 4°C.

Blocking Buffer & Antibodies

- 11 Determine how many controls you will be using. Typically we have four tubes:

	Name	Antibody	Fluorophore	Volume
	Control 1	NeuN	PE	0.5 μ L
	Control 2	Olig2	Alexa 488	0.4 μ L
	Isotype Control	IgG	PE	0.5 μ L
	Isotype Control	IgG	Alexa 488	0.5 μ L

The amount of Blocking Buffer we will need to resuspend our nuclei pellet with after centrifuging depends on the amount of tubes we need. Each tube will require 50 μ L of nuclei suspension, and our sample needs to have 1,000 μ L of nuclei suspension left over. So in this case we will need $1000\mu L + (50\mu L \times 4) = 1200\mu L$ of Blocking Buffer for resuspending the nuclei pellet.

- 12 Add 930 μ L Blocking Buffer to each of the control tubes.
- 13 After the cell homogenate is done centrifuging, discard the supernatant and resuspend the pellet in 1200 μ L of Blocking Buffer. Incubate for 15 minutes on ice.
- 14 Add 50 μ L of nuclei suspension to each of the four control tubes.
- 15 Add the corresponding antibodies to the control tubes:
- 0.5 μ L NeuN-PE
 - 0.4 μ L Olig2-Alexa 488
 - 0.5 μ L IgG-PE
 - 0.5 μ L IgG-Alexa 488
- 16 Add 0.5 μ L NeuN-PE and 0.4 μ L Olig2-Alexa 488 to the sample tube.
- 17 Place all tubes in a rotator placed in a 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 *If there is no visible pellet* after centrifugation, remove 950 µL of supernatant with a small pipette being careful not to disturb the bottom of the tube. Then resuspend the pellet in 950 µL Blocking Buffer.

If there is a visible pellet, remove all of the supernatant without disturbing the pellet and then resuspend in 930 µL of Blocking Buffer.
- 20 Add 20 uL Hoechst to each sample and control tube and gently mix by tapping.
- 21 Transfer all samples and controls to 2 mL tubes that will be less forceful on nuclei during FACS.
- 22 Store tubes on ice until FACS. Bring Hoechst stock with you to FACS.

Protocol references

Adapted from the Allen Institute's protocol.