Nuclei from Suspension Cells

Protocol Overview

- Start with Viable Cell Enrichment (VCE) to remove dead cells and debris from single-cell suspension.
- Perform nuclei extraction by directly adding Nuclei Isolation Buffer (NIB) to the enriched live cells and incubating on ice.
- Wash samples to remove ambient nucleic acid and prep for downstream assay of choice.

Tips

1. For suspension cells, nuclei extraction is done by incubating the cells in NIB on ice for 5 mins so the pestle and tubes will not be needed.
2. Buffers can be made while cells are levitating during Viable Cell Enrichment.
3. Each suspension cell nuclei extraction reaction should have <2 million cells to prevent clumping.

Kit Components

Reagents	Quantity (16 rxn)	Quantity (4 rxn)	Shipping Conditions	Storage Conditions	Prep for use
10X Buffer N3	1 x 6 mL	1 x 6 mL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component L	1 x 600 µL	1 x 600 µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component A[1]	2 x 1400 µL	1 x 1400 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw at RT and place on ice
Component R[1]	1 x 350 µL	1 x 350 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw on ice
Particle M	4 x 55 µL	1 x 55 µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Equilibrate to RT for use
2X Buffer WB	1 x 75 mL	1 x 75 mL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice

[1] Components A and R need to be transferred to -20°C immediately upon arrival. Components A and R are not stable with long term storage at 2-8°C .

Consumables	Quantity (16 rxn)	Quantity (4 rxn)	Shipping Conditions	Storage Conditions
.5 mL tubes (sterile/RNase free)	16 ea	4 ea	Ambient Temperature	Store at room temperature
Pestles (sterile/RNase Free)	16 ea	4 ea	Ambient Temperature	Store at room temperature
40 µm Filters	16 ea	4 ea	Ambient Temperature	Store at room temperature

Prepare Buffers

Prepare Nuclei Isolation Buffer (NIB) - Store on Ice

Prepare Nuclei Isolation Buffer for the number of samples being processed in a single day according to Table 1 below. For more than 3 samples, a 5 mL tube is recommended. Label this "NIB." Store on ice throughout the protocol. Once prepared, NIB should be used within the day. Mix via inversion or by pipetting after all components have been added.

Table 1. Volumes used to prepare Nuclei Isolation Buffer (includes overage)

Reagent	Volume for 1 sample (µL)	Volume for 4 samples (µL)
10X Buffer N3	51	204
Nuclease-Free Water	455	1820
Component L	6	24
Component R	3	12
Component A[1]	58	232
RNaseOUT[2]	3	12
Total	**576**	**2304**

Tips

1. Component A should be stored at -20°C immediately after arrival. Once thawed (on ice), it can be stored at 2-8°C for up to 72 hrs or refrozen twice. Buffers can be made while cells are levitating during Viable Cell Enrichment.
2. RNaseOUT is not supplied with the kit and can be substituted with the RNase Inhibitor of the user's choice. Final RNase Inhibitor concentration should be at 0.2 U/µL.

Prepare 1X Suspension Wash Buffer (1X SWB) - Store on ice

Table 2. Reagent volumes used to prepare SWB master mix (includes overage)

Reagent	Volume for 1 sample (µL)	Volume for 4 sample (µL)
10X Buffer N3	220	880
Component R	11	44
RNaseOUT (Final Conc 0.2 U/µL)	11	44
Nuclease-Free Water	1958	7832
Total	**2200**	**8800**

Protocol

! Note: *Buffers can be made while cells are levitating during Viable Cell Enrichment.*

1. Nuclei extraction from suspension cells starts by enriching for viable cells.
   a. VCE is recommended for all samples regardless of starting viability.
   b. Please follow guidelines on VCE using the LeviCell or EOS.
2. Extract nuclei using the 500 µL of NIB.

! Note: *For downstream assays that require a Digitonin step during sample prep, spike the appropriate amount of Digitonin into the NIB Buffer per downstream assay prior to nuclei extraction.*

   a. If a sample was split across multiple VCE runs due to the total cell number, it can be pooled for nuclei extraction, keeping the number of live cells < 2 million cells and the total volume < 200 µL.
   b. Pipette mix sample with NIB (5-10X at a rate of 2X per second). Do NOT vortex.

! Note: *For very limited samples, dispense NIB directly above the levitated sample and mix gently and thoroughly by tapping the tube. This will aid in not losing samples to the pipette tip.*

   c. Incubate on ice for 5 min.
3. Pellet nuclei extraction in a pre-chilled (4°C) centrifuge at 500 RCF for 3 minutes.
4. Remove and discard supernatant.
5. Add 1000 µL of 1X SWB to each sample. Ensure the pellet is resuspended.
6. Spin sample in a pre-chilled (4°C) centrifuge at 500 RCF for 3 minutes.
7. Remove and discard supernatant.

! Note: *Carefully pipette away any bubbles first, then with a fresh pipette tip remove the remaining supernatant. In a swinging bucket centrifuge the pellet will be at the bottom of the tube in a single location; in a fixed angled centrifuge, the nuclei might be along the entire wall of the tube so positioning is extremely important to know where your sample will be. In either case, remove supernatant away from where sample is expected to be and tip tube to remove maximum volume instead of moving pipette tip to remove excess buffer.*

8. Perform final wash using 1 mL of the recommended buffer for the downstream kit being used. If no recommended buffer is mentioned for downstream reaction, 1X SWB can be used.

   i.e. For 10X Genomics Multiome or ATAC-Seq chemistry, pipette mix nuclei with 1 mL of ice cold 1X Nuclei Buffer (From 10X Genomics ATAC or Multiome Kit) for the 2nd (final) wash. For 3' snRNAseq chemistry, pipette mix nuclei with 1 mL of ice cold 1X SWB.

9. Spin sample in a pre-chilled (4°C) centrifuge at 1000 RCF for 3 minutes.

10. Remove and discard supernatant. (Refer to note at step 7.)

11. Resuspend sample in the appropriate buffer for your downstream application. 50 to 100 µL is recommended for resuspension unless downstream application requires extremely concentrated samples. For samples with low cell numbers resuspend in residual 1X Buffer within the tube.

Additional guidelines for final resuspension

- Users should determine what low cell number entails based on the concentration desired for downstream activity and the starting concentration of the sample.
- Keep in mind dilution is easier than concentrating with an additional spin.
- Be sure to properly dilute samples for counting to be within the range of the counting device being used.
- If there are excessive nuclei clumps, the sample can be passed through a 40 µm filter. Be sure to count the sample again prior to proceeding with single cell sequencing.