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Protocol for nuclei isolation from fresh and frozen tissues using Salty-Ez10 buffer: compatible with snRNA-Seq and Multiome workflows from 10x Genomics V.1

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In Development dx.doi.org/10.17504/protocols.io.buxknxkw

Human Cell Atlas Method Development Community | Single Cell Core, Harvard Medical School

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ABSTRACT

This is a protocol in development, which means it has not yet tested/challenged with multiple samples. So, please make sure you take it for a test drive before committing to it. Once you do please share your experience with me via email, Twitter or as a comment.

EXTERNAL LINK

https://singlecellcore.hms.harvard.edu/

DOI

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PROTOCOL CITATION

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KEYWORDS

snRNAseq, 10x, nuclei, isolation, FACS

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GUIDELINES

Use RNA techniques.

The use of RNAse Inhibitor is HIGHLY recommended.

MATERIALS TEXT

Required Buffers and Reagents

1. Salty-Ez10 Lysis buffer

10 mM Tris-HCl pH 7.5
146 mM NaCl
1 mM CaCl2
21 mM MgCl2
0.03% Tween-20
0.01% BSA (Miltenyi, 130-091-376)
10% Ez Lysis Buffer (Sigma)
0.2-1 U/uL Protector RNAse Inhibitor (*yes, use this one*!)

2. Wash and Resuspension Buffers

Wash and Resuspension Buffer 1 (WRB1)

1x PBS
1% BSA (Molecular Grade)
0.2-1 U/uL Protector RNAse Inhibitor

Wash and Resuspension Buffer 2 (WRB2)

10 mM Tris-HCl pH 7.5
10 mM NaCl
3 mM MgCl2
1% BSA
0.2-1 U/uL Protector RNAse Inhibitor

For Multiome (10x Genomics) use Digitonin (Themo, BN2006).

For cell counting: Acridine Orange/Propidium Iodide (AO/PI) Cell Viability Kit (F23001)

3. Gradient Solutions

G60: OptiPrep®: 60% (w/v) solution of iodixanol in water.

GD: 150 mM KCl, 30 mM MgCl2, 120 mM, Tricine-KOH pH 7.8.

GW: 50% of iodixanol --> mix 5 volumes of G60 with 1 volume of GD (final: 50% iodixanol, 25 mM KCl, 5 mM MgCl2, 20 mM Tricine-KOH pH 7.8).

GH: 0.25 M Sucrose, 25 mM KCl, 5 mM MgCl2, 20 mM Tricine-KOH pH 7.8. (keep at 4°C).

G30: solutions of 30% --> mix 6 volumes of GW with 4 volumes of GH (final: 30% iodixanol, 25 mM KCl, 5 mM MgCl2, 20 mM Tricine-KOH pH 7.8). **Prepare fresh.**

4. Stock solutions and GD/GH Set-Up

-500 mM Tricine: 8.96 g in 100 ml water
-1 M KCl: 7.45 g in 100 ml water
-1 M MgCl2x6H2O: 20.3 g in 100 ml water

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GD: To 50 ml water add 24 ml, 15 ml and 3 ml respectively of the Tricine, KCl and MgCl2•6H2O stock solutions (above); adjust to pH 7.8 with 1 M KOH and make up to 100 ml. Keep at RT.

GH: Dissolve 8.5 g of sucrose in 50 ml of water; add 4 ml, 2.5 ml and 0.5 ml respectively of the Tricine, KCl and MgCl2•6H2O stock solutions (above); adjust to pH 7.8 with 1 M KOH and make up to 100 ml. Keep at 4°C.

SAFETY WARNINGS

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

DISCLAIMER:

BEFORE STARTING

All samples and reagents are kept on ice or at 4 °C (wet ice).

Prepare all buffers and reagents as described in the "Materials" section.

Tissue Homogenization

1 Mince/chop tissue with a razor blade to small pieces. The tissue may be as small as a grain of rice.

For mincing the tissue, you may take the tube out of ice, however, be quick and return it to ice.

2 Add **300 µl** of chilled Salty-Ez10 Lysis Buffer (supplemented with RNAse Inhibitor 0.2-0.5 U/uL) to the tissue in 1.5 mL tube.

3 **Gently** homogenize the sample using a douncer by stroking 10-20 times. Keep nuclei suspension on ice at all times.

4 Add an extra **700 µl** of chilled Salty-Ez10 Lysis Buffer (supplemented with RNAse Inhibitor 0.2-0.5 U/uL), mix gently by pipetting using **wide-bore tips** and incubate on ice for **00:05:00** . Repeat mixing 2-3 times during the incubation. 5m

Lysis Optimization can be done using the Luna-FL™Dual Fluorescence Cell Counter (this is the best automatic

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counter to me and makes Countess look like a Random Number Generator!) and by taking aliquots at different times until you get less than 5% viability using Acridine Orange/PI dual staining.

Nuclei Isolation 10m

5 Filter homogenate using a 70 µm-strainer mesh to fit a **pre-cooled** 15 ml Falcon tube (e.g. pluriStrainer Mini 70 µm Cell Strainer). This step is to remove undigested tissue or fat prior to centrifugation.

6 Transfer flow though into a 1.5 mL LoBind tube and centrifuge the nuclei at **500 x g** for **00:05:00** at **4 °C** (these 5' count as lysis time too!). Remove supernatant leaving behind ~ **50 µl** if the pellet seems loose.

7 **Optional:** add **1 mL** of Salty-Ez10 Lysis Buffer (supplemented with RNAse Inhibitor 0.2-if pellet is loose0.5 U/uL), 10m gently resuspend pellet (optional: incubate for **00:05:00** on ice for additional lysis). Then centrifuge the nuclei at **500 x g** for **00:05:00** at **4 °C** .

Nuclei Wash and Resuspension 10m

8 ⚠

After removing the supernatant, add **500 µl** of WRB1 (supplemented with RNAse Inhibitor 0.2-0.5 U/uL) **without disturbing the pellet**. Let sit for 5' on ice and then gently resuspend the pellet (this incubation is important to avoid clumping).

--> ALTERNATIVELY, resuspend the pellet in WRB2 (supplemented with RNAse Inhibitor 0.2-0.5 U/uL). The 5' incubation is optional for this buffer. **WRB2 is my favorite so far**.

Warning: some nucs preps clump when you add WRB1 (this is a known phenomenon with Tris to PBS enchange, hence 5' on ice helps but not always! It's sample dependent). In that case use WRB2 (my favorite). Both WRBs are compatible with all 10x kits.

Important: for 10x Multiome (10x Genomics) add 0.01% Digitonin in the WRB buffers (supplemented with RNAse Inhibitor 0.2-0.5 U/uL), not during lysis step itself. In this way the time the nuclei is leaky (due to holes caused by Digitonin) is shorter, 5' on ice is enough to permeabilize nuclei. **Then replace it by the 1x Nuclei Buffer (NB) as indicated in the user guide** (this buffer is essential for ATAC or Multiome kit).

9 If cell debris and large clumps are observed, pass through a cell strainer. For low volume, use a 40 µm Flowmi Cell Strainer to minimize volume loss.

If high amounts of debris or micelles (fat) is present you may perform OptiPrep® ***continuous*** gradient as done in

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DOF (dx.doi.org/10.17504/protocols.io.bs99nh96, steps 9-12). Use WRBs for washes and resuspension for snRNA-Seq. For Multiome use WRBs for washes and resuspension 1x NB for resuspension. Pass through a cell strainer (e.g., 40 µm Flowmi Cell Strainer).

10 Check integrity and purity under microscope and count manually or using an automatic counter. For automatic counter I recommend Luna-FL™ Dual Fluorescence Cell Counter and Acridine Orange/Propidium Iodide (AO/PI) Cell Viability Kit. This instrument also provides information about size and multiplets which is very useful.

11 Proceed to your amazing snRNA-Seq or Multiome experiment!

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