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Nuclei isolation from human frozen adipose tissue for single-nuclei RNA-sequence. Adapted from three published articles. V.2

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Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

This is a protocol for isolating nuclei from human adipose tissue and it was adapted from three published articles.

Nuclei isolation from human frozen adipose tissue for single-nuclei RNA-sequence

1 **First essential step**

1. Freshly prepare all reagents on the day of isolation.
2. Wipe all surfaces and equipment with RNAse Zap
3. Make sure the centrifuge is at 4° C

2 **Buffer Preparation**

2x ST (It can be prepared previous evening and kept at room temperature)

	A	B	C	D
	Reagent	**Stock Concentration**	**2X ST Buffer Concentration**	**Volume for 50 mL of 2X ST Buffer**
	NaCl	5 M	292 mM	2.92 mL
	Tris	1 M	20 mM	1 mL
	CaCl2	1 M	2 mM	100 µL
	MgCl2	1 M	42 mM	2.1 mL
	H2O	-	-	43.88 mL

TST (2 mL should be prepared for each tissue sample) (homogenization buffer)
*10% Tween-20 can be prepared ahead of time and stored at 4ºC

	A	B
	Reagent	**Volume for 2 mL Working Solution (per Sample)**
	2x ST stock solution	1000 µL
	BSA Stock Solution (10%)	2 µL (0.01% final concentration)
	10% Tween-20*	6 µL
	H2O	636 µL
	Superase RNase Inhibitor (20U/µL)	20 µL (1 U/µL final concentration)

	A	B
	Sucrose (1.5M)	334 ul
	Protease inhibitor (100x)	2 ul

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1x ST

	A	B
	Reagent	**Volume for 3.5 mL Working Solution (per Sample)**
	2x ST stock solution	1748 µL
	H2O	1717 µL
	Ribolock RNase Inhibitor (40 U/µL)	35 µL

PBS + BSA (1%) + RNase Inhibitors

	A	B
	Reagent	**(per Sample)**
	PBS 1X	3500 µL
	BSA Stock Solution (10%)	400 µL
	Protector RNase Inhibitor (40 U/µL)	100 µL

4 **Tissue Dissociation**

- Fill a GentleMACS C tube with 2 mL of TST buffer per sample. Keep tubes on wet ice.
- Transfer a ~130-150mg piece of adipose tissue directly into the buffer.
- Make sure that tissues are floating freely in buffer and not sticking onto the walls of the tube.

- Secure the C tubes to the GentleMACS Dissociator and run the “mr_adipose tissue, 35 sec” program.
- Repeat the program for three runs on each tissue sample.
- Detach the tubes from the GentleMACS Dissociator and Incubate the samples on wet ice for 10 minutes
  (upside down).
- Set the acceleration to 10 and deceleration to 5
- Centrifuge the tubes for 2 minutes at 500g at 4ºC to collect the suspension and remove any foam.
- Immediately after centrifugation, resuspend the nuclei pellet in the supernatant within the
  same C tube. Pipette up and down 30 times.

3. **Filtration**

- Place a 50 mL Falcon tube on wet ice with a 40 um Falcon Cell Strainer.
- First wash the filter with 1 mL of 1x ST buffer with RNase inhibitors.
- Transfer the dissociated ~2 mL suspension of nuclei into the filter.
- Wash the used C tube with 1 mL of 1x ST buffer with RNase inhibitors, then transfer it also into the
  filter.
- Lastly, wash the filter with an additional 1 mL of 1x ST with RNase inhibitors.

4. **Centrifugation and nuclei isolation**

- Centrifuge in a swinging bucket rotor for 10 minutes at 2500 g and 4ºC.
- Carefully remove the supernatant by pipetting, leaving approximately 20 µL behind in the tube.
- Wash the nuclear pellet 2-3 times with 1000 µL of PBS + BSA + RNase Inhibitor solution. Gently pipette up and down 30 times before each centrifugation.
- Resuspend the remaining nuclear pellet in 100 µL of PBS + 1% BSA + RNase Inhibitor solution.
- Submit the nuclei sample immediately for QC and cDNA preparation.

Adapted from three published articles.

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