Dec 11, 2024 Version 1

Nuclei isolation from human frozen adipose tissue for single-nuclei RNA-sequence. Adapted from two published articles. V.1

DOI
dx.doi.org/10.17504/protocols.io.eq2ly6w5qgx9/v1

Mythili Dileepan[1], David Bernlohr[1], Paul Robbins[1], Laura Niedernhofer[1]

[1]University of Minnesota

Cellular Senescence Net...

Mythili Dileepan
University of Minnesota

DOI: https://dx.doi.org/10.17504/protocols.io.eq2ly6w5qgx9/v1

Protocol Citation: Mythili Dileepan, David Bernlohr, Paul Robbins, Laura Niedernhofer 2024. Nuclei isolation from human frozen adipose tissue for single-nuclei RNA-sequence. Adapted from two published articles.. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.eq2ly6w5qgx9/v1

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

Created: December 11, 2024

Last Modified: December 11, 2024

Protocol Integer ID: 115047

Keywords: nuclei from human visceral adipose tissue, human frozen adipose tissue, isolating nuclei, human visceral adipose tissue, nuclei rna, rna, nuclei isolation from human frozen adipose tissue, nuclei isolation, nuclei, tissue

Funders Acknowledgements:
NIH Common Fund
Grant ID: U54 AG076041

Abstract

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

Troubleshooting

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

	A	B
	Superase RNase Inhibitor (20U/µL)	20 µL (1 U/µL final concentration)
	Sucrose (1.5M)	334 ul
	Protease inhibitor (100x)	2 ul

3

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 a total of three runs on each tissue sample.
- Detach the tubes from the GentleMACS Dissociator.
- 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
  foam.
- Immediately following the centrifugation, resuspend the nuclei pellet in the supernatant
  within the same C tube. Pipet up and down 30 times.

3. **Filtration**

- Prepare a 50 mL Falcon tube on wet ice with a 40 um Falcon Cell Strainer.
- Wash the filter with 1 mL of 1x ST buffer with RNase inhibitors.
- Transfer the homogenized ~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 into the filter.
- 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 at 4ºC.
- Carefully remove the supernatant by pipetting out
- Wash the nuclear pellet 2-3 times in 1000ul of “pbs+BSA+ RNAse Inhibitor solution “. Pipet up and down 30 times gently before centrifuge.
- Resuspend the remaining nuclei pellet in 100 µL of PBS + BSA (1%) + RNase Inhibitors solution.
- Submit the nuclei sample immediately for QC and cDNA preparation.

Adapted from two published articles.

5

1. Emont, M.P., Jacobs, C., Essene, A.L. *et al.* A single-cell atlas of human and mouse white
adipose tissue. *Nature* **603**, 926–933 (2022). https://doi.org/10.1038/s41586-022-04518-2

2. Whytock KL, Divoux A, Sun Y, Hopf M, Yeo RX, Pino MF, Yu G, Smith SR, Walsh MJ, Sparks LM. Isolation of nuclei from frozen human subcutaneous adipose tissue for full-

length single-nuclei
transcriptional profiling. STAR Protoc. 2023 Mar 17;4(1):102054. doi:
10.1016/j.xpro.2023.102054. Epub 2023 Jan 20. PMID: 36853719; PMCID:PMC9876942.