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Nuclei Isolation from Human Brain Using Sucrose Gradient

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Protocol status: Other
We attempted this protocol, but could not get it to work in our group

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Abstract

This protocol outlines our preparation of single-nuclei suspension from surgically acquired fresh human adult brain tissue.

1 Prepare Nuclei Suspension Buffer (NSB)

For drop-seq: 1x PBS, 0.01% BSA (ultra pure), 0.2U/ul RNAse inhibitor.

For 10X Genomics: 1X PBS, 1%BSA (ultra pure), 0.2U/ul RNase inhibitor

2 Prepare lysis buffer and sucrose solution:

	Lysis buffer	**30 ml**	**Final**
	2M Sucrose	4.8 ml	0.32 M
	1M $CaCl_2$	150 µl	5 mM
	1M $Mg(Ac)_2$	90 µl	3 mM
	0.5M EDTA	6 µl	0.1 mM
	1M Tris-HCl(pH8.0)	300 µl	10 mM
	0.1M PMSF	30 µL	0.1 mM
	100% Triton X-100	30 µL	0.10%
	100% NP-40	30 µL	0.10%
	Protease inhibitor	300 µL	1X
	RNase inhibitor	150 µL	0.1 U/µL
	Molecular biology grade (MBG) water	24.26 ml	

	Sucrose solution	**50 ml**	**Final**
	2M sucrose	45 ml	1.8 M
	1M $Mg(Ac)_2$	150 µl	3 mM
	1M Tris-HCl(pH8.0)	500 µl	10 mM
	MBG water	4.35 ml	

3 Put ~250mg of brain tissue into a douncer with 10ml of ice-cold lysis buffer on ice.

4 Dounce the tissue for 2 min while on ice.

5 Take 18ml of sucrose solution into a new 30ml clear ultracentrifuge tube on ice.

6 Place the homogenized brain into ultracentrifuge tube on ice (gradient with the homogenized brain on top of the sucrose solution).

7 Weigh the ultracentrifuge tubes, and adjust the weight by lysis buffer.

8 Ultracentrifuge with SW28 rotor at 24,400RPM (=107,163.6 RCF) for 2.5h at 4°C.

9 Remove supernatant, add 500µL of NSB to each pellet, and incubate them on ice for 10min.

10 Resuspend the nuclei in NSB, filter through Flowmi cell strainer (Bel-Art, H13680-0040) and transfer the suspension into one 2ml tube on ice.

11 Stain 10µl of nuclei suspension with DAPI (1:5000) or 0.4% Trypan Blue to count.

12 Adjust the volume with NSB to 1000 nuclei/µl (for 10X Genomics) and 300 nuclei/µl (for drop-seq).