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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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Nuclei Isolation from Human Brain Using Sucrose Gradient

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Human Cell Atlas Method Development Community

Neurodegeneration Method Development Community

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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
2 M sucrose	45 ml	1.8 M
1 M Mg(Ac) ₂	150 µl	3 mM
1 M Tris-HCl (pH 8.0)	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 ($\approx 107,163.6$ RCF) for 2.5h at 4°C.
- 9 Remove supernatant, add 500 μ L 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 10 μ L of nuclei suspension with DAPI (1:5000) or 0.4% Trypan Blue to count.
- 12 Adjust the volume with NSB to 1000 nuclei/ μ L (for 10X Genomics) and 300 nuclei/ μ L (for drop-seq).