Nuclei Isolation from Human Brain Using Sucrose Gradient

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Abstract

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

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Protocol

Step 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

Step 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	

Step 3.

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

Step 4.

Dounce the tissue for 2 min while on ice.

Step 5.

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

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

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

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

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

Step 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.

Step 11.

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

Step 12.

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