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Nuclei Isolation from Fresh Frozen Human Brain “Villages”

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We use this protocol and it's working

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Abstract

This protocol describes nuclei isolation from fresh frozen human brain tissue, using anywhere from 1 brain donor to ~20-25 brain donors in one prep.

Materials

Reagents to prepare (keep all on ice):

- Dounce homogenizer
- 1.5mL and 50mL tubes
- Nuclei EZ lysis buffer, Sigma Cat #NUC101-1KT
- RNase inhibitor, NxGen 30281-1 OR Ribolock EO-0384
- Phosphate Buffered Saline solution (PBS)
- G60 (OptiPrep®: 60% (w/v) solution of iodixanol in water (92339-11-2))
- BSA powder
- 500mM Tricine
- 1M potassium chloride (KCl)
- 1 M magnesium chloride ($MgCl_2 \times 6H_2O$)
- Potassium hydroxide (KOH)
- pH indicator strips
- Sucrose

Before start

Prepare the described stock solutions prior to beginning your brain dissections.

Procedure

1 Place a clean 7 mL dounce on ice.

1.1 *** Can use a 14 mL dounce if using >50 mg tissue per donor x20 donors, or 2 mL dounce for n=1-4 donors.*

2 Add ~3 mL of the total 7 mL chilled EZ lysis buffer + RNase inhibitor to the dounce. Pour frozen tissue carefully into the dounce. Use the remaining EZ lysis buffer to wash residual tissue out of the tube/container, as well as from the sides of the dounce.

3 Gently dounce tissue anywhere from 5 to 20 times with pestle “A” or “loose” depending on the quantity and quality of the tissue. For white matter rich regions, dounce very gently. The tissue can still look slightly chunky after pestle A. Next, carefully dounce 5-20 times with pestle “B” or “tight” until just homogenized.

3.1 *** Optimize protocol by eye to ensure you are using the least number of dounce cycles as possible for the region and tissue you are working with while still producing a homogenous solution. You may still see particles after pestle B but they are likely white matter and will be filtered out later.*

4 Incubate the homogenized solution for 10 minutes on ice in the dounce.

5 Prewet a 20 µm vacuum filter with 1 mL "PBSAi", then pour dounce contents ½ at a time over the filter and apply gentle vacuum, trying to avoid creating a lot of bubbles. Use a second filter if it clogs, collecting the pull through into one 50 mL conical.

6 Centrifuge at 4°C for 5 min (500 x g) in filter conical, using large tabletop centrifuge.

7 Remove and discard supernatant from the 50ml conical.

8 Resuspend pellet in ~1 mL G30. If the resuspension looks thick or very milky with white matter debris, add more G30 (up to 3 mL) until it has thinned out.

8.1 *** For every 1 mL used to resuspend the pellet, you will need ~9 mL more of G30 to separate into the spin tubes for the next section. This will vary by tissue input.*

9 Prepare an appropriate number of 1.5 mL snap cap tubes by filling each with 1 mL of G30 (ex: about 9 tubes for 1mL of resuspended pellet). While tilting the tube slightly, gently overlay 300 µL of the resuspended sample by slowly flowing it down the side of the tube very gently, so that the layers do not mix.

10 Centrifuge the 1.5ml tube samples at 8000 x g, 10 minutes minimum (up to 20 minutes), 4°C.

11 Remove and discard supernatant, aspirating from the top of the liquid line to remove all floating debris layers.

12 Resuspend each pellet in ~50 µL PBSAi, being careful not to wash down any cellular debris "junk" that may have stuck to the sides of the tube, then combine all into one new 1.5 mL tube. Add PBSAi up to the 1.5 mL mark on the tube and pipette mix gently but thoroughly.

13 Centrifuge at 4°C for 5 min (500 x g).

14 Remove and discard supernatant.

15 Resuspend pellet in ~100µL PBSAi; adding more or less according to pellet size.

15.1 *** We tend to start with 100-200 µL, immediately make a 1:10 dilution by adding 10 µL nuclei to 90 µL PBSAi, and count the diluted sample.*

16 Count nuclei using LUNA-FL counter.

17 Combine 8 µL PBSA + 1 µL AO-PI dye + 1 µL resuspended nuclei (dilution factor = 9). Use 10 µL to load in each side of slide.

18 To preserve leftover nuclei, centrifuge nuclei at 4°C for 5 min (500g) if you have a large amount (> 1 million nuclei). Skip centrifuge if you have less than 1 million nuclei.

18.1 Remove supernatant and add CryoStor at <=1000 nuclei/µL and mix by pipetting.

18.2 Transfer to cryotubes and store in -80°C freezer indefinitely.

Preparing stock solutions

19 Basic solutions:
500 mM tricine: 8.96 g in 100 mL water.
1 M KCl: 7.45 g in 100 mL water.
1 M MgCl2×6H2O: 20.3 g in 100 mL water.

20 "PBSA": 1% BSA in 1X PBS**:** 0.01g BSA / 1 mL 1X PBS, pH 7.4.
“PBSAi”: Add 125uL of RNase inhibitor (40U/uL, 1U/uL final) to 5mL of PBSA.

21 **GD**: Add 24 mL tricine, 15 mL KCl, 3 mL MgCl2×6H2O to 50 mL water. Adjust to pH 7.8 with 1 M KOH and make up to 100 mL. Keep at room temperature.
GH: Dissolve 8.5 g of sucrose in 50 mL of water. Add 4 mL tricine, 2.5 mL KCl and 0.5 mL MgCl2×6H2O. Adjust to pH 7.8 with 1 M KOH and make up to 100 mL. Keep at 4°C.
G30: combined 6mL of G60 ("Optiprep") with 1.2mL of GD. Mix thoroughly. Lastly, add 4.8mL of GH.

22 Nuclei EZ lysis buffer + RNase inhibitor (1 U/uL final): combined 7mL of EZ Lysis buffer with 175uL of RNase inhibitor (40 units per uL) per village.