Oct 29, 2021

Human_Tissue_Nuclei_Isolation_Protocol_2021_10_18

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dx.doi.org/10.17504/protocols.io.bzmgp43w

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Homebrew protocol to isolate nuclei from human frozen brain tissue

DOI

dx.doi.org/10.17504/protocols.io.bzmgp43w

Jose.Bras , lee.marshall , Kimberly Paquette 2021. Human_Tissue_Nuclei_Isolation_Protocol_2021_10_18. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bzmgp43w

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Stock Solutions

10% Triton X-100 (100mL)

Solution	Final Conc	Volume	Notes
Triton X-100	10%	10mL	
Ultrapure Water		Fill to: 100mL	

1M MgCl2 (100mL)

Solution	Final Conc	Volume	Notes
MgCl2 (FW 203.31)	1M	20.331g	203.31g/1000mL = 1M 5.08275g/100mL = 1M
Ultrapure Water		Fill to: 100mL	

Working Solutions

*Use PBS without Calcium Chloride and Magnesium Chloride

*PBSTA (3mL) without TritonX-100 – store at 4*C for up to 1 month*

Solution	Final Conc	1 RXN	5.2 RXN
10x PBS	1x	300uL	1560uL
1M MgCl2	3mM	9uL	46.8uL
D-Sucrose (342.29)	0.3M	0.3081g	1.602g
**** RNasein (add on day)**	0.4 U/uL	30uL	156uL
Ultrapure Water		Fill to: 3mL	Fill to: 15.6mL

- Add most of the Ultrapure water, then PBS, then MgCl2, then Sucrose
- Vortex to dissolve
- Then add Ultrapure water to final volume
- Add RNasein before using

1.4M PBS Cushion (8mL) – make fresh weekly

Solution	Final Conc	1 RXN	5.2 RXN
10x PBS	1x	0.8mL	4.16mL
1M MgCl2	3mM	24uL	124.8uL
D-Sucrose	1.4M	3.8336g	19.935g
10% Triton X-100	0.1%	80uL	416uL
Ultrapure Water		Fill to: 8mL	Fill to: 41.6mL

- Add half of the Ultrapure water, then PBS, then MgCl2, then Sucrose
- Vortex to dissolve
- Then add 10% Triton,
- Then add Ultrapure water to final volume

Nuclei Wash and Resuspension Buffer – make fresh daily

Solution	Final Conc	1 RXN	5.2 RXN
10x PBS	1x	400uL	2080uL
50mg/uL Ultrapure BSA	1%	800uL (40mg)	4160uL
**** RNasein (add on day)**	0.2 U/uL	20uL	104uL
Ultrapure Water		Fill to: 4mL	Fill to: 20.8mL

- Add most of the Ultrapure water, then PBS, then BSA
- Tilt to dissolve, do not vortex

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- Add RNasein before using

1 Homogenize
a. Transfer tissue to facs tube
b. Add 2mL of PBSTA to the facs tube containing the tissue
c. Homogenize on ice with hand-held mixer at lowest setting, 5 sec on, 5 sec off, repeat for a total of 4 times, (do not create bubbles, check no debris after homogenizing), store on ice
d. Add 40uL of 10% TX100 (final conc 0.2%), pipette mix 10 times with p1000, incubate for 5min on ice. For each sample leave 2min space between
e. Repeat for each sample a-c for each sample

2 Extract nuclei and filter onto gradient
a. Transfer the 2mL of PBSTA/tissue to a Dounce on ice
b. Dounce 10 times gently to release the nuclei, do a half turn at the top and bottom movement, do not create bubbles by never lifting the Dounce out of the volume at all
c. Filter the nuclei through a Miracloth (Cakbiochem, #475855) onto the 8mL of 1.4M PBS Cushion
d. Add 1mL of PBSTA to the Dounce to resuspend any remaining nuclei from the sides of the dounce, transfer these through the same Miracloth onto the 1.4M PBS Cushion
e. Repeat for each sample a-e for each sample

3 Sucrose Gradient Centrifuge
a. Do not disturb the gradient
b. Centrifuge nuclei through the gradient @ 3000 x g for 30 min at 4*C
c. Take out of centrifuge immediately when finished, should see a condensed white interphase

4 Resuspend Nuclei
a. On ice, remove the top layer first using a p1000
b. Then remove the interphase (cell debris) using a p1000
c. Once the cell debris is removed, continue to use a p1000 to remove all supernatant, switch to a p200 tip to remove the final 200uL supernatant, make sure to remove all the supernatant without touching the pellet
d. Resuspend the pellet in 210uL of Nuclei Wash and Resuspension Buffer, pipette 10 times using a p200, then transfer to LoBind tube on ice.
e. Further resuspend the nuclei into singlets by pipette 5 times using a p200 against the wall of the epi
f. Repeat for each sample a-e for each sample

5 Count Nuclei
a. Transfer 10uL of nuclei into a fresh epi on ice
b. Add 10uL of Trypan Blue into the 1:2 nuclei dilution, resuspend 10 times and count nuclei numbers using a Countess

6 Wash Nuclei 1
a. After incubation, add 900mL of Nuclei Wash Buffer to the 200uL nuclei, pipette 5 times with p1000
b. Centrifuge nuclei @ 500 x g for 5min at 4*C
c. Discard most of the supernatant using a p200 tip, leaving 20uL of supernatant and pellet, being

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careful not to disturb the pellet
d. Pipette 10 times using a p200 to resuspend the pellet

7 Wash Nuclei 2
a. Add 1000uL of Nuclei Wash Buffer to the 100uL nuclei, pipette 5 times with p1000
b. Centrifuge nuclei @ 500 x g for 5min at 4*C
c. Discard most of the supernatant using a p200 tip, leaving 20uL of supernatant and pellet, being careful not to disturb the pellet
d. Pipette 10 times using a p200 to resuspend the pellet

8 Wash Nuclei 3
a. Add 1000uL of Nuclei Wash Buffer to the 100uL nuclei, pipette 5 times with p1000
b. Centrifuge nuclei @ 500 x g for 5min at 4*C
c. Discard most of the supernatant using a p200 tip, leaving 20uL of supernatant and pellet, being careful not to disturb the pellet
d. Pipette 10 times using a p200 to resuspend the pellet
e. Add 100uL of Nuclei Wash Buffer, pipette 10 times using a p200 to resuspend the pellet

9 Count Nuclei
a. Add 5uL of nuclei to 5uL of Nuclei Wash Buffer in a fresh epi on ice
b. Add 10uL of Trypan Blue resuspend 10 times and count nuclei numbers using a Countess and take photos on the EVOS level 3

10 Flow cytometry
a. Add 20uL of nuclei to 180uL of Nuclei Wash Buffer in a fresh epi on ice
b. Flow QC and Count Nuclei Dilution Protocol

11 Dilute Nuclei to 1000 nuclei/uL
a. Add 100,000 nuclei into 100uL of Nuclei Wash Buffer Snap Freeze leftover Nuclei Protocol

12 Snap Freeze leftover nuclei
b. Use liquid nitrogen to snap freeze leftover nuclei and store in -80 freezer

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