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Human_Tissue_Nuclei_Isolation_Protocol_2021_10_18

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Protocol status: Working

We use this protocol and it's working

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Abstract

Homebrew protocol to isolate nuclei from human frozen brain tissue.

Attachments



[401-868.pdf](#)

51KB

Materials

Stock Solutions

- 10% Triton X-100 (100mL)
- 1M MgCl₂ (100mL)

Working Solutions

- PBS
- RNasein
- MgCl₂
- D-Sucrose
- Ultrapure Water
- Ultrapure BSA



Stock Solutions

1 10% Triton X-100 ( 100 mL)

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

2 [M] 1 Mass Percent MgCl_2 ( 100 mL)

	A	B	C	D
	Solution	Final Conc	Volume	Notes
	MgCl_2 (FW 203.31)	1 M	20.331 g	$203.31 \text{ g}/1000 \text{ mL} = 1 \text{ M}$ $5.08275 \text{ g}/100 \text{ mL} = 1 \text{ M}$
	Ultrapure Water		Fill to: 100 mL	

Working Solutions: PBSTA (3mL) without TritonX-100

3

Note

*Use PBS without Calcium Chloride and Magnesium Chloride.

Note

Note: Store at  4 °C for up to 1 month.





A	B	C	D
Solution	Final Conc	1 RXN	5.2 RXN
10x PBS	1x	300 uL	1560 uL
1M MgCl ₂	3 mM	9 uL	46.8 uL
D-Sucrose (342.29)	0.3 M	0.3081 g	1.602 g
** RNasein (add on day)	0.4 U/uL	30 uL	156 uL
Ultrapure Water		Fill to: 3 mL	Fill to: 5.6 mL

Add most of the Ultrapure water, then PBS, then MgCl₂, then Sucrose.

4 Vortex to dissolve.

5 Then add Ultrapure water to final volume.

6 Add RNasein before using.

Working Solutions: 1.4 M PBS Cushion (8 mL)

7

Note

Note: Make fresh weekly.

A	B	C	D
Solution	Final Conc	1 RXN	5.2 RXN
10x PBS	1x	0.8 mL	4.16 mL
1 M MgCl ₂	3 mM	24 uL	124.8 uL
D-Sucrose	1.4 M	3.8336 g	19.935 g



	A	B	C	D
	10% Triton X-100	0.10%	80 uL	416 uL
	Ultrapure Water		Fill to: 8 mL	Fill to: 41.6 mL

Add half of the Ultrapure water, then PBS, then MgCl_2 , then Sucrose.

8 Vortex to dissolve.

9 Then add 10% Triton.

10 Then add Ultrapure water to final volume.

Working Solutions: Nuclei Wash and Resuspension Buffer

11

Note

Note: Make fresh daily.

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

Add most of the Ultrapure water, then PBS, then BSA.

12 Tilt to dissolve, do not vortex.



13 Add RNasein before using.

