Jun 25, 2024

Human_Tissue_Nuclei_Isolation_Protocol_2021_10_18

DOI
dx.doi.org/10.17504/protocols.io.5qpvoboy7l4o/v1

Kimberly Paquette[1]

[1]Center for Alzheimer's and Related Dementias, National Institute on Aging, Bethesda, Maryland, USA

Team Lee

Jane Balster
ASAP - Team Lee

DOI: dx.doi.org/10.17504/protocols.io.5qpvoboy7l4o/v1

Protocol Citation: Kimberly Paquette 2024. Human_Tissue_Nuclei_Isolation_Protocol_2021_10_18. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5qpvoboy7l4o/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 11, 2022

Last Modified: June 25, 2024

Protocol Integer ID: 60564

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 $MgCl_2$ (100mL)

Working Solutions

- PBS
- RNasein
- $MgCl_2$
- 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 Molarity (M) $MgCl_2$ (100 mL)

	A	B	C	D
	Solution	Final Conc	Volume	Notes
	MgCl2 (FW 203.31)	1 M	20.331 g	203.31 g/1000 mL = 1 M 5.08275 g/100 mL = 1 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

	A	B	C	D
	1M MgCl2	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 $MgCl_2$, 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 MgCl2	3 mM	24 uL	124.8 uL
	D-Sucrose	1.4 M	3.8336 g	19.935 g
	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.