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RNA Extraction from Cultured Human Dorsal Root Ganglia

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PRECISION Human Pain ...

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

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Abstract

This protocol was developed to perform RNA extraction from cultured hDRG neurons using Qiagen RNeasy Plus Micro kit

Materials

	Material	Company	Product Number
	RNAeasy Plus Micro Kit	Qiagen	74034
	DPBS	Cytiva	SH30264.FS
	gDNA Eliminator Spin Column	Qiagen	1030958
	RLT Plus Buffer	Qiagen	1030963
	Beta Mercaptoethanol	Fisher Scientific	BP176-100
	100% Ethanol	Fisher Bioreagents	BP2818-500
	MinElute Spin Columns	Qiagen	1026497
	RW1	Qiagen	1015763
	RNAse Free DNAse Set	Qiagen	79256
	Reconstituted DNAse	Qiagen	1010395
	RDD Buffer	Qiagen	1010397
	RPE	Qiagen	1017974
	Ultrapure Distilled Water	Invitrogen	10977-015
	Cell Scraper	Fisher Scientific	08-100-241
	1.7mL Microcentrifuge Tube	Costar	3621
	2.0mL Collection Tube	Qiagen	1016810

Troubleshooting

Safety warnings

Safety training courses on Bloodborne Pathogens, Biological Safety, Chemical Hygiene, Compressed Gases, Biological Waste Management, Personal Protective Equipment, Autoclave and Media Kitchens, General Laboratory Safety, and Cryogen Safety are required by UTD and can be taken through its BioRAFT system.

Safety Training related to Biomedical Research with Human Subjects is required by UTD and can be taken through its CITI program.

Ethics statement

All human tissue recovery, research and data handling is performed in accordance with the University of Texas at Dallas Institutional Review Board (IRB) regulations.

Preparation

1 Cell cultures are prepared as described in **dx.doi.org/10.17504/protocols.io.kxygxyd64l8j/v1**

2 Prepare RLT Plus containing Beta mercaptoethanol in separate tube in fume hood

2.1 Prepare 350 ul of RLT Plus lysis buffer for each sample

2.2 Add Beta mercaptoethanol 1:100 in RLT Plus Buffer

2.3 Example: Add 10 ul of Beta mercaptoethanol to 1ml of RLT Plus

2.4 Vortex

3 Prepare 70% ETOH by diluting 100% ETOH in Ultrapure Distilled Water

4 Prepare 80% ETOH by diluting 100% ETOH in Ultrapure Distilled Water

RNA Extraction

5 Aspirate media and wash cells 1x with 500ul of 1X DPBS

6 Performing one well at a time, aspirate out DPBS and add 350ul of RLT plus buffer with Beta mercaptoethanol to each well

6.1 Be careful when pipetting as this is soapy and will form a lot of bubbles if not careful

7 Scrape down cells using cell scraper and collect RLT plus buffer and place in 1.5ml tube

7.1 Vortex all samples for 30 seconds

8 Transfer the lysate to a gDNA eliminator spin column place in a 2ml collection tube

8.1 Centrifuge for 30 seconds at ≥8000g at room temperature

8.2 Discard column and SAVE flow-through

9 Add 1 volume of 70% ETOH (usually around 350ul) to the flow-through and mix well by pipetting

10 Transfer the sample to an RNeasy MinElute spin column placed in 2ml collection tube

10.1 Centrifuge for 15 seconds at ≥8000g at room temperature

10.2 Discard flow-through

11 Add 350 ul of RW1 to RNeasy MinElute spin column

11.1 Centrifuge for 15 seconds at ≥8000g at room temperature

11.2 Discard flow-through

12 DNAse clean up step

12.1 Add 70ul of RDD buffer to 10ul aliquots of reconstituted DNase

12.2 Add 80ul of RDD buffer + DNase directly to spin column

12.3 Incubate for 15 minutes at room temperature

13 Add 350 ul of RW1 to RNeasy MinElute spin column

13.1 Centrifuge for 15 seconds at ≥8000g at room temperature

13.2 Discard flow-through

14 Add 500 ul of RPE RNeasy MinElute spin column

14.1 Centrifuge for 15 seconds at ≥8000g at room temperature

14.2 Discard flow-through

15 Add 500 ul of 80% ETOH to RNeasy MinElute spin column

15.1 Centrifuge for 2 minutes at ≥8000g at room temperature

15.2 Discard flow-through and collection tube

16 Place MinElute spin column in new 2ml collection tube

16.1 Open lid of spin column

16.2 Centrifuge for 5 minutes at full speed (16,000g) at room temperature

16.3 Discard flow-through and collection tube

17 Place MinElute spin column in new 1.5ml collection tube

18 Place 14ul of DNA/RNA free water directly to center of spin column

18.1 Incubate for 1 minute at room temperature

18.2 Centrifuge for 1 minute at full speed (16,000g)

18.3 Keep collection tube, and discard column

19 Freeze and store RNA by placing in -80°C freezer