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Disc Tissue RNA Extraction and Qubit Measurement

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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

A protocol for RNA extraction from human lumbar disc tissue.

Guidelines

Collection of tissue for this protocol requires prior approval by the user's Institutional Ethics Board or equivalent ethics committee.

Materials

	A	B	C
	Material	**Company**	**Product Number**
	CP02 cyroPREP Automated Dry Pulverizer	Corvaris	500001
	tissueTUBE TT1 Extra Thick	Corvaris	520007
	1.7mL Microcentrifuge Tube	Costar	3621
	Ultrapure Distilled Water	Invitrogen	10977-015
	RNAzap	Invitrogen	AM9780
	Kimwipes	Kimtech	06-666A
	Qiazol	Qiagen	1023537
	Chloroform	Fisher Scientific	C298-500
	100% Ethanol	Fisher Bioreagents	BP2818-500
	RNeasy Spin Column	Qiagen	1112543
	Buffer RW1	Qiagen	1015763
	RNAse Free DNAse Set	Qiagen	79256
	Reconstituted DNAse	Qiagen	1010395
	RDD Buffer	Qiagen	1010397

	A	B	C
	Buffer RPE	Qiagen	1017974
	2.0mL Collection Tube	Qiagen	1016810
	Qubit RNA HS Assay Kit	Invitrogen	Q32855
	Qubit RNA HS Reagent (200X)	Invitrogen	Q32855 A
	Qubit RNA HS Buffer	Invitrogen	Q32855 B
	Qubit RNA HS Standard (0ng/uL)	Invitrogen	Q32855 C
	Qubit RNA HS Standard (10ng/uL)	Invitrogen	Q32855 D
	Qubit Assay Tubes	Invitrogen	Q32856

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.

Store Tissue in RNAlater

1 Isolate nucleus pulposus (NP) and annulus fibrosus (AF) tissue and freeze immediately in powdered dry ice or place in 5ml tube with 4ml of RNAlater

2 If in RNAlater place in 4°C refrigerator overnight

3 Next day remove tissue from RNAlater and freeze in powdered dry ice

4 Place in new tube and store in -80°C

Extract RNA

5 Remove tissue, cut off a section, and place in a 1.5 mL tube on dry ice. Measure the weight of each tissue.

5.1 Less than 100mg, the smaller the better, especially for AF samples

5.2 Prechill tubes to try and keep tissue as frozen as possible

6 Place tissue pieces in Corvaris TT2 bag and leave on dry ice till ready to pulverize

7 Dip bag into liquid nitrogen and impact with Corvaris until powder is formed

7.1 It is important to move frozen debris around after each impact to thoroughly pulverize

8 Add 1 mL Qiazol to bag to retrieve frozen, pulverized samples, and place back in 1.5ml tube

9 Add 200 uL of chloroform to each tube

9.1 Shake for 15 seconds and let incubate for 2 minutes at room temp.

10 Centrifuge at 4°C 12,000 g, 10 minutes

11 Collect upper aqueous phase. Transfer it to a new microfuge tube.

11.1 Add again 200 uL of chloroform to aqueous phase, shake, incubate, and centrifuge.

12 Collect upper aqueous phase. Transfer it to a new microfuge tube.

12.1 Add again 200 uL of chloroform to aqueous phase, shake, incubate, and centrifuge.

13 Collect upper aqueous phase. Transfer it to a new microfuge tube.

14 Add same volume of 70% RNAse free ethanol (~300-400 uL).

14.1 Mix gently.

15 Precipitate on ice for 10 minutes.

16 Load 700 uL of sample onto RNeasy spin column

16.1 Centrifuge 15 sec >8,000 g at room temperature.

16.2 Discard the flow through.

16.3 Repeat until loading until the whole sample has gone through the column.

17 Add 350 uL Buffer RW1 to the RNeasy spin column.

17.1 Centrifuge 15sec >8,000 g at room temperature.

17.2 Discard the flow through and collection tube.

18 Perform DNAase clean up step

18.1 Add 70 ul of buffer RDD (fridge) to aliquots of Qiagen DNAse (10 ul) (-20°C)

18.2 Add the total volume from tube (80 ul) directly on to column

18.3 Incubate at room temperature for 15 minutes

19 Add 350 uL Buffer RW1 to the RNeasy spin column.

19.1 Centrifuge 15sec >8,000 g at room temperature.

19.2 Discard the flow through and collection tube.

20 Perform DNAase clean up step a second time

20.1 Add 70 ul of buffer RDD (fridge) to aliquots of Qiagen DNAse (10 ul) (-20°C)

20.2 Add the total volume from tube (80 ul) directly on to column

20.3 Incubate at room temperature for 15 minutes

21 Add 350 uL Buffer RW1 to the RNeasy spin column.

21.1 Centrifuge 15sec >8,000 g at room temperature.

21.2 Discard the flow through and collection tube.

22 Pipette 500 uL Buffer RPE onto the RNeasy spin column.

22.1 Centrifuge 15sec >8,000 g at room temperature.

22.2 Discard the flow through.

23 Pipette 500 uL Buffer RPE onto the RNeasy spin column.

23.1 Centrifuge 2 min > 8,000 rpm at room temperature.

23.2 Discard the flow through.

24 Transfer RNeasy column to new 2mL collection tube

24.1 Centrifuge for 1 minute to dry collection tube at full speed (16,000g) at room temperature.

25 Transfer the RNeasy column to 1.5 mL collection tube

25.1 Pipette 15 uL RNase-free water; incubate 1 minute.

25.2 Centrifuge RNeasy column > 8,000 g, at room temperature for 15 seconds.

25.3 Pipette the same 15 uL RNase-free water in collection tube back onto column; incubate 1 minute.

25.4 Centrifuge RNeasy column > 8,000 g at room temperature for 1 minute.

26 Measure RNA concentration with Qubit

26.1 Prep working reagent
i. 200 ul per tube
ii. 1 tube for each sample
iii. 2 tubes for standards
iv. 200x stock solution at room temperature
v. Rest of contents should be at 4°C
vi. Need to use on Qubit tubes

26.2 Prep standards
i. 10 ul of low standard + 190 ul working reagent
ii. 10 ul of high standard + 190 ul working reagent

26.3 Prep samples
i. 1-5 ul of sample + 199-195 ul of working reagent

26.4 Vortex and read samples on Qubit machine