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RNA Isolation from Human Intervertebral Disc Tissue

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RE-JOIN

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We use this protocol and it's working

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Abstract

Isolation of high-quality RNA from intervertebral disc (IVD) tissue is challenging due to its dense extracellular matrix, high proteoglycan content, and relatively low cell density. This protocol presents an optimized method for RNA extraction separately from the annulus fibrosus (AF) and nucleus pulposus (NP). The procedure incorporates mechanical dissociation, TRIzol™-based homogenization, multiple phenol–chloroform extractions, and purification using the Qiagen RNeasy Lipid Tissue Mini Kit. This kit was chosen over the Universal RNeasy Mini Kit due to the importance of the RW1 buffer. For RNA isolation from NP tissue, a larger amount of starting tissue (200 mg) was used as compared to AF tissue (50 mg) due to the lower cellularity and higher ECM content of NP, which typically yields less RNA per mg of tissue. To avoid overloading the RNA-binding capacity of individual RNeasy spin columns and to ensure efficient RNA recovery, the lysate from NP tissue was divided across four columns, whereas only one column was sufficient for the smaller AF tissue sample. This protocol yields RNA of high quality that is suitable for downstream applications such as RNA-sequencing and qPCR.

Guidelines

This protocol needs prior approval by the users' Institutional Review Board (IRB) or equivalent ethics committee(s).

Materials

1. Qiagen RNeasy Lipid Tissue Mini Kit (50) (Cat. # 74804) including Buffer RW1 and Buffer RPE
2. Allprotect® Tissue Reagent (Qiagen)
3. 5 mL Eppendorf Tube® (Cat. # EP0030119487) for freezing tissue in Allprotect®
4. Phosphate-buffered saline (PBS)
5. Liquid nitrogen (LN_2)
6. TRIzol™ Reagent (Invitrogen)
7. Chloroform
8. 70% RNase-free ethanol
9. DNase I (Zymo)
10. DNA digestion buffer (Zymo)
11. RNase-free water
12. Kimwipes
13. 1.5 mL Eppendorf Tube® (Sigma; Cat. # EP022363531)
14. 2 mL Eppendorf Tube® (Sigma; Cat. # EP022600044)
15. Tissue Pulverizer (Cell Crusher; Cat. # NC1824866)
16. Spatula
17. Vortex mixer
18. Microcentrifuge
19. NanoDrop™ 1000 (NanoDrop Technologies)
20. TapeStation System (Agilent Technologies)

Troubleshooting

Tissue Resection, Storage, and Homogenization

1 Tissue collection and storage

1.1 Human lower thoracic and entire lumbar spines obtained from an organ procurement organization should be processed within 48 hours post-mortem.

1.2 After resecting IVDs from the spine, separate AF and NP, cut into smaller pieces and immediately place them in 4 ml of Allprotect® in 5 ml Eppendorf tubes and store at 4°C overnight, then place into -80°C for long-term storage.

2 Removal of Allprotect®, weighing, and tissue preparation

2.1 Thaw the tissue.

2.2 Gently remove residual Allprotect® using a Kimwipe and briefly rinse the tissue with PBS.

2.3 Cut off a section from the tissue of approximately target amount. For AF, use ~50 mg; for NP, ~200 mg and weigh. Cut additional slice as necessary.

2.4 Snap-freeze the tissue in liquid nitrogen and transfer it to a new tube on dry ice (or keep in LN_2) until further processing. NOTE: Scale up subsequent reagent volumes proportionally for NP. The same pulverization and homogenization procedure applies to both AF and NP. Lysate from NP tissue was divided across four columns after the ethanol precipitation step.

3 Mechanical pulverization

3.1 Pre-cool the tissue pulverizer (cell crusher) with LN_2.

3.2 Place the frozen tissue into the pre-cooled cell crusher, add LN_2, and pulverize to a fine powder using a hammer.

3.3 Rotate the cell crusher after each impact for thorough pulverization.

3.4 Continue until a fine powder is achieved. This procedure should be done quickly so that the cell crusher and tissue do not warm up.

4 Homogenization in TRIzol™

4.1 Transfer the pulverized tissue to a 2 mL tube containing 1 mL of ice-cold TRIzol™ using a clean spatula.

4.2 Vortex for 20 seconds, then place on ice for 1 minute. Repeat 2 more times.

4.3 Incubate the samples on a rotator at 4°C for 20 minutes.

4.4 Centrifuge at 12,000 rpm at 4°C for 30 seconds.

4.5 Transfer the supernatant (~1 mL) to a new pre-cooled 2 mL tube and store at −80°C or proceed to the next step.

Phenol–Chloroform Extraction

5 First extraction

5.1 Add 200 µL of chloroform to the homogenate in TRIzol™.

5.2 Shake vigorously for 15 seconds and incubate for 2 minutes at room temperature (RT).

5.3 Centrifuge at 12,000 rpm at 4°C for 10 minutes.

5.4 Carefully collect the upper aqueous phase and transfer it to a new microcentrifuge tube.

6 Second extraction

6.1 Add 200 µL of chloroform to the aqueous phase.

6.2 Shake, incubate for 2 minutes at RT, and centrifuge at 12,000 rpm at 4°C for 10 minutes.

6.3 Collect the upper aqueous phase and transfer it to a new microcentrifuge tube.

7 Third extraction

7.1 Add 200 µL of chloroform to the aqueous phase.

7.2 Shake, incubate for 2 minutes at RT, and centrifuge at 12,000 rpm at 4°C for 10 minutes.

7.3 Collect the final upper aqueous phase and transfer it to a new microcentrifuge tube.

RNA Extraction Using the Qiagen RNeasy Lipid Tissue Mini Kit

8 Ethanol precipitation

8.1 Add an equal volume (1:1) of 70% RNase-free ethanol (e.g., 300–400 µL) to the aqueous phase.

8.2 Mix gently and incubate on ice for 10 minutes.

9 Binding to the RNeasy column

9.1 Load 700 µL of the sample onto an RNeasy spin column.

9.2 Centrifuge for 15 seconds at >8,000 rpm at RT; discard the flow-through.

9.3 Repeat until the entire sample has passed through the column.

9.4 Note: For NP samples divide the sample into four parts and load onto four RNeasy spin columns to prevent clogging.

10 First wash

10.1 Add 350 µL of Buffer RW1 to the spin column.

10.2 Centrifuge for 15 seconds at >8,000 rpm at RT; discard the flow-through and the collection tube.

11 DNase treatment (first round)

11.1 Prepare the DNase reaction by adding 70 µL of DNA digestion buffer (Zymo) to 10 µL of Zymo DNase I (total 80 µL).

11.2 Pipette the entire 80 µL directly onto the column membrane.

11.3 Incubate for 15 minutes at RT.

12 Second wash

12.1 Add 350 µL of Buffer RW1 to the spin column.

12.2 Centrifuge for 15 seconds at >8,000 rpm at RT; discard the flow-through and the collection tube.

13 DNase treatment (second round)

13.1 Prepare another DNase reaction: add 70 µL of DNA digestion buffer (Zymo) to 10 µL of Zymo DNase I (80 µL total).

13.2 Pipette the 80 µL directly onto the column membrane.

13.3 Incubate for 15 minutes at RT.

14 Third wash

14.1 Add 350 µL of Buffer RW1 to the spin column.

14.2 Centrifuge for 15 seconds at >8,000 rpm at RT; discard the flow-through and the collection tube.

15 Buffer RPE washes

15.1 Transfer the spin column to a new collection tube.

15.2 Add 500 µL of Buffer RPE.

15.3 Centrifuge for 15 seconds at >8,000 rpm at RT; discard the flow-through.

15.4 Add another 500 µL of Buffer RPE.

15.5 Centrifuge for 2 minutes at >8,000 rpm at RT; discard the flow-through.

16 Final drying spin

16.1 Place the RNeasy column in a new 2 mL collection tube.

16.2 Centrifuge for 2 minutes at full speed (16,000 × g) at RT to dry the membrane thoroughly.

17 Elution and storage

17.1 Transfer the RNeasy column to a 1.5 mL microcentrifuge tube.

17.2 Add 15 µL of RNase-free water to the membrane and incubate for 1 minute.

17.3 Centrifuge at >8,000 rpm for 15 seconds at RT.

17.4 Return the same 15 µL eluate onto the column membrane, incubate for 1 minute, and centrifuge again for 1 minute at >8,000 rpm at RT.

17.5 Store the eluted RNA at −80°C until use.

Assessment of RNA Concentration, Purity, and Integrity

18 Spectrophotometric analysis

18.1 Apply 1 µL of RNA to a NanoDrop™ 1000 (NanoDrop Technologies) to measure RNA concentration and purity.

19 Integrity analysis

19.1 Use 2 µL of RNA on the 4150 TapeStation System (Agilent Technologies) to determine RNA Integrity Number (RIN).