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RNA Isolation of Human Osteochondral, Subchondral Bone and Cartilage Tissues

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Protocol status: Working
We use this protocol and it's working very well for RNAseq.

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Disclaimer

Abstract

This protocol demonstrates how to perform RNA isolation from fresh snapfrozen Human Osteochondral Tissue, Subchondral Bone and Cartilage tissues.

Materials

Liquid Nitrogen (LN)
Ladle for Liquid Nitrogen
Wet Ice in Ice Bucket
Dry Ice to keep samples frozen
RNAse Away spray bottle to clean tools and workspace
Clean Mortar and Pestle
Silicone Cover for Mortar and Pestle
Small plastic scoop
Clean forceps to take out sample from tube
Kim Wipes
Scalpel for tissue cutting
Trizol
Petri dish
2.0mL microtubes
Benchtop vortex
Microtube rotator: 4℃ walk-in refrigerator
Precooled 4℃ benchtop centrifuge
70% Ethanol
Chloroform
100% Ethanol - to make complete RWT and RPE buffer IF NEEDED
Qiagen RNeasy Plus Universal Kit - columns,
collection tubes, 1.5mL microtubes, gDNA Eliminator, complete RWT buffer,
complete RPE buffer, DNase free water

Tissue Homogenization

1 Add 1 mL Trizol to Eppendorf tubes and place tubes on ice. For each Trizol-filled microtube, place another empty 2.0mL tube on ice.

2 Precool Benchtop Centrifuge and make sure temperature is at 4°C.

3 Prepare tools, mortar and pestle, and work surface by spraying down with RNAse Away.

4 Weigh out 110-150mg of fresh or snapfrozen tissue. If you have to cut a smaller piece of snapfrozen tissue to fit weight range, do so in a petri dish placed on a piece of dry ice so sample remains frozen.

5 Add two ladles of liquid nitrogen to a clean mortar and pestle; allow to become cold.

6 After about half of liquid nitrogen has evaporated, add snapfrozen tissue to the mortar.

7 Add another ladle of liquid nitrogen to mortar.

8 Begin smashing and grinding up tissue with the pestle; this is an ongoing process so keep adding liquid nitrogen until grinding creates a fine powder. Do not let the mortar and sample get warm.

8.1 Note - samples have a tendency to pop out of the mortar, having a silicone lid top on the mortar in the beginning helps keep tissue contained.

9 Once you have the desired consistency of a fine powder, add half a ladle of liquid nitrogen and tilt mortar slightly on its side and begin scraping liquid nitrogen/powdered tissue suspension to the bottom for a more thorough collection.

10 Just as the liquid nitrogen evaporates, use a pre-chilled scoop to transfer tissue powder right away to a chilled 2.0mL microtube with Trizol.

11 Use a benchtop vortex for 20 seconds max speed then place on ice for 1 minute. Repeat 2 times for a total of 3 times so that no clumps remain.

12 Place tube on Rotator at 4°C and rotate for 20 minutes.

13 After 20 minutes, spin for 1 minute in precooled benchtop centrifuge at 12,000g at 4°C

14 Transfer supernatant to pre-chilled 2.0mL microtube.

15 You may continue OR stop here and immediately place sample into a -80°C freezer for next day RNA extraction.

RNA Extraction Using Qiagen RNeasy Plus Universal Mini Kit

16 Precool Benchtop Centrifuge to 4°C AND make sure you have another benchtop centrifuge at Room Temperature.

17 Gradually thaw samples on wet ice if previously frozen at -80°C.

18 To each trizol tube: Add 111uL gDNA Eliminator, vortex for 15 seconds. Add 220uL Chloroform, vortex for 30 seconds, and set out at Room Temperature for 3 minutes.

19 Spin at 12,000g for 15 minutes in 4°C benchtop centrifuge.

20 Transfer top aqueous layer to a Room Temperature 2.0mL microtube. Be careful to not disturb the middle and bottom layers.

21 Add 1:1 70% Ethanol to the tube with top aqueous layer. (For example- 600uL top layer and 600uL 70% Ethanol). Mix well by pipetting up and down.

22 Allow the top aqueous layer/ethanol mixture to come to Room Temperature.

23 Place 600uL into the Qiagen Column with the collection tube. When loading column, be careful to not get the rim wet. Do not load in more than 650uL of mixture to the column at a time.

24 Spin at 12,000 rpm in Room Temperature benchtop centrifuge.

25 Discard flow through after spin, invert collection tube and dab tube on paper towel to remove excess liquid.

26 Repeat steps 23, 24, and 25 until all of the mixture has gone through the column.

27 Place tubes in rack and open tops for 2 minutes to allow columns to air dry and remaining ethanol to evaporate. This improves 260/230 ratio.

28 Add 700uL of RWT buffer to each column.

29 Spin for 15 seconds at 12,000 rpm in Room Temperature centrifuge. Discard flow through.

30 Add 500uL of RPE buffer to each column, incubate at Room Temperature for 5 minutes.

31 Spin for 15 seconds at 12,000 rpm in Room Temperature centrifuge. Discard flow through.

32 Repeat steps 30 and 31 two additional times, for a total of 3 RPE washes.

33 Spin for 2 minutes at 12,000 rpm in Room Temperature centrifuge.

34 Place column in new collection tube.

35 Spin-dry for 3 minutes at 12,000 rpm Room Temperature centrifuge.

36 Place column in new 1.5mL Eppendorf tube.

37 Add 30uL water to column to elute RNA.

38 Spin for 1 minute at 12,000 rpm in Room Temperature centrifuge

39 Place eluate back into column and spin once more for 1 minute at 12,000 rpm.

40 Check RNA concentration using Nanodrop.