Aug 16, 2024

RNA Isolation of Human Meniscus

DOI
dx.doi.org/10.17504/protocols.io.8epv5ropdg1b/v1

Takuya Sakamoto[1], Merissa Olmer[1], Martin Lotz[1]

[1]Scripps Research

RE-JOIN

Merissa Olmer
Scripps Research

DOI: dx.doi.org/10.17504/protocols.io.8epv5ropdg1b/v1

Protocol Citation: Takuya Sakamoto, Merissa Olmer, Martin Lotz 2024. RNA Isolation of Human Meniscus. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5ropdg1b/v1

Protocol status: Working
We use this protocol and it's working for bulk RNA isolation and RNA-seq.

Created: August 16, 2024

Last Modified: August 16, 2024

Protocol Integer ID: 105853

Keywords: Musculoskeletal, Human, Knee, Meniscus, RNA isolation, RNA

Funders Acknowledgement:
RE-JOIN
Grant ID: AR082186 02

Disclaimer

Abstract

This protocol demonstrates how to perform RNA isolation from fresh snapfrozen and AllProtect preserved human meniscus tissue.

Materials

Liquid Nitrogen (LN)
Ladle for Liquid Nitrogen
Wet ice in Ice Bucket
Dry Ice to keep samples frozen
RNAse Away reagent to clean tools and workspace
Clean Mortar and Pestle
Silicone Cover for Mortar and Pestle
Small plastic scoop (chemical scoop)
Clean forceps to take out sample from tube
Scalpel for tissue cutting
Kimwipes
Trizol or TRI Reagent
CK28R Precellys Ceramic Bead Tubes
Precellys or Bead Tissue Disruptor
1.5 mL microtubes
Benchtop vortex
Microtube rotator in 4C walk in fridge
Precooled 4C benchtop centrifuge
100% Ethanol for protocol and to make complete prewash and wash buffers IF NEEDED
Chloroform
Zymo Direct-zol RNA MicroPrep Kit - columns, collection tubes, prewash buffer, wash buffer, DNase I, DNA Digestion Buffer, DNase free water

Before start

This protocol works with both fresh snapfrozen and AllProtect preserved tissue.

Tissue Homogenization

1 Add 1 mL Trizol/TRI Reagent to CK28R bead tubes and place tubes 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 100mg of preserved tissue. If you have to cut a smaller piece of tissue to get 100mg, do so in a petri dish placed on a piece of dry ice so sample remains frozen.

4.1 If sample was stored in AllProtect, briefly rinse in PBS and wipe off excess liquid with Kimwipes prior to weighing out tissue.

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 or AllProtect tissue to the mortar.

7 Add another ladle of liquid nitrogen to mortar and allow about half of the liquid nitrogen to boil off.

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 into a chilled CK28R tube with Trizol.

11 Place cooled CK28R tubes with samples in the Precellys and use 6500rpm, 20 second cycles for a total of 4 times. Cool tubes on ice for 3 minutes between each cycle.

12 Once samples are finished on the Precellys with 4 cycles run, transfer Trizol to a new 1.5mL tube.

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

RNA Extraction Using Zymo Direct-zol MicroPrep Kit

14 Preparation before starting the isolation protocol:
i. Pre-cool Benchtop Centrifuge to 4°C AND make sure you have another benchtop centrifuge at Room Temperature.
ii. Prepare Digestion Buffer (DNaseI 5ul x__+1__, DNA Digestion Buffer 35ul x__+1__) according to Zymo's protocol.

15 Add 200uL chloroform (20% volume) to the Trizol and vortex for 30 seconds. If Trizol samples were previously frozen at -80°C, gradually thaw samples on wet ice.

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

17 Transfer top aqueous layer (about 400uL) to a Room Temperature 1.5mL microtube. Be careful to not disturb the middle and bottom layers.

18 Add 80uL (20% volume) of chloroform, vortex for 30 seconds. Incubate tubes for 10 minutes at Room Temperature.

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

20 Transfer 400uL of the aqueous phase into 1.5mL microtube.

21 Add 1:1 of 100% Ethanol to the tube with aqueous phase. (For example- 400uL top layer and 400uL 100% Ethanol).

22 Mix Ethanol and aqueous phase gently by pipetting and then add 400uL to Zymo column. When loading column, be careful to not get the rim wet.

23 Centrifuge at 14,000 rpm for 1 minute at 4°C.

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

25 Repeat Steps 22-23 so that all of the mixture has gone through the column.

26 Add 400uL of Direct-zol RNA Wash Buffer to the column and centrifuge at 14,000 rpm for 1 minute at 4°C.

27 Discard flow-through.

28 Add 40uL Digestion Buffer (see step 14 ii.) to the column matrix. Incubate at Room Temperature for 15 minutes.

29 Add 400uL of Direct-zol RNA PreWash Buffer to the column and centrifuge at 14,000 rpm for 1 minute at 4°C.

30 Discard flow-through.

31 Repeat Steps 28 and 29.

32 Add 700uL of Direct-zol RNA Wash Buffer to the column and centrifuge at 14,000 rpm for 1 minute at 4°C.

33 Discard flow-through and the collection tube. Put the column in a new collection tube.

34 Centrifuge for 2 minutes at 14,000 rpm at 4°C.

35 Transfer the column carefully into a 1.5mL tube.

36 Add 15uL of DNase/RNase-Free Water to the membrane to elute RNA. Centrifuge at 14,000 rpm for 1 minute at 4°C.

37 Check RNA concentration using Nanodrop.