Sep 23, 2025

Muscle Tissue RNA Extraction

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6zxo1gqe/v1

Joesph B Lesnak[1], Theodore Price[1]

[1]University of Texas at Dallas

PRECISION Human Pain ...

Camryn Wellman
University of Texas at Dallas

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6zxo1gqe/v1

Protocol Citation: Joesph B Lesnak, Theodore Price 2025. Muscle Tissue RNA Extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6zxo1gqe/v1

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

Created: August 21, 2025

Last Modified: September 23, 2025

Protocol Integer ID: 225196

Keywords: rna extraction from human lumbar muscle tissue, muscle tissue rna extraction, human lumbar muscle tissue, rna extraction

Funders Acknowledgements:
NIH
Grant ID: U19NS130608

Abstract

A protocol for RNA extraction from human lumbar muscle 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 TT05M XT Extra Thick	Corvaris	520140
	1.7mL Microcentrifuge Tube	Costar	3621
	Ultrapure Distilled Water	Invitrogen	10977-015
	RNAzap	Invitrogen	AM9780
	Kimwipes	Kimtech	06-666A
	Buffer RLT	Qiagen	1015750
	β-mercaptoethanol (BME)	Fisher Scientific	BP176-100
	Proteinase K	Qiagen	19131
	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

	A	B	C
	RDD Buffer	Qiagen	1010397
	Buffer RPE	Qiagen	1017974
	2.0mL Collection Tube	Qiagen	1016810

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.

Prep Work

1 Remove tissue, cut off a section, and place in a 1.5 ml tube on dry ice. Measure the weight of each tissue and write down

1.1 Roughly 30-50 mg of tissue

1.2 Try to avoid really blood sections

1.3 Can be stored in tubes long term till ready to extract

2 Prepare Lysis buffer

2.1 300 ul needed per sample

2.2 Add 10 ul of β-mercaptoethanol (BME) per 1 mL of Buffer RLT

3 Turn on heat block to 55°C

Extraction RNA

4 Place tissue pieces in Corvaris TT1 bag and leave on dry ice till ready to pulverize

5 Dip bag into liquid nitrogen and impact with Corvaris till powder is formed

5.1 Pulverize all samples before continuing

6 Add 300 uL of Buffer RLT + BME lysis buffer to bag to retrieve frozen, pulverized samples, and place back in 1.5 mL tube

6.1 Mix up and down with pipette tip to get all powdered sample around bag

7 Add 590 uL of DNA/RNAse free water to each sample

8 Add 10 uL of proteinase K to each sample

9 Mix with hand by turning over and place in heat block at 55°C for 15 minutes

10 Centrifuge sample for 10,000g at room temp for 3 minutes

11 Transfer supernatant to new tube

12 Add 0.5 volume of 100% ETOH (~450 ul) to each sample and mix by hand, do not vortex

13 Load 700 uL of sample onto RNeasy spin column

13.1 Centrifuge for 15 seconds >8,000g at room temperature.

13.2 Discard the flow through.

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

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

14.1 Centrifuge for 15 seconds >8,000g at room temperature.

14.2 Discard the flow through.

15 Perform DNAase clean up step

15.1 Add 70 uL of Buffer RDD (fridge) to aliquots of Qiagen DNAse (10 uL) (-20°C)

15.2 Add the total volume from tube (~80 uL) directly on to column

15.3 Incubate at room temperature for 15 minutes

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

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

16.2 Discard the flow through.

17 Add 500 uL Buffer RPE onto RNeasy spin column

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

17.2 Discard the flow through.

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

18.1 Centrifuge 2 minutes > 8,000g at room temperature.

18.2 Discard the flow through.

19 Transfer RNeasy spin column to new 2 mL collection tube

20 Centrifuge RNeasy spin column for 1 minute to dry at full speed (16,000g) at room temperature.

21 Transfer the RNeasy column to new 1.5 mL collection tube

21.1 Pipette 40 uL of RNase-free water; incubate 1 min.

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

22 Measure concentration and A260/A280 and A260/A230 ratios on nanodrop

23 Store RNA in -80°C