



GeneLab Standard Operating Procedure: Qiagen AllPrep DNA/RNA Mini with Qiagen RNase-Free DNase Set

May 2020

Version 1.0



Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-3.1	1.0	May 2020	Original document

Scope and Purpose

This procedure describes the steps required to extract both RNA and DNA from mammalian tissues. In general, the expected RNA yield when using this procedure with the homogenization methods described in SOP #2.1 and SOP #2.2 is 0.2 - 0.4 uG of RNA per each mG of tissue homogenized. The extraction kit used in this procedure is Qiagen AllPrep DNA/RNA Mini. In addition, in this procedure we describe steps for depleting the isolated RNA from DNA using QIAGEN RNase-Free DNase set. This step is required for RNA that will be used for sequencing. It is strongly advised to read the AllPrep DNA/RNA Mini-Handbook in full. List of validated tissues:

1. spleen
2. eye
3. adrenal gland
4. liver
5. kidney
6. thymus
7. lung
8. colon
9. brain
10. white adipose
11. brown adipose
12. lymph nodes
13. reproductive tract
14. muscles (Appendix A)
15. heart (Appendix A)
16. skin tissue (Appendix A)

Some buffers in this kit contain guanidine salt and are not compatible with disinfectants containing bleach.



Equipment and Consumables

1. Eppendorf Centrifuge 5424/5424 R
2. Bench top microcentrifuge to accommodate 1.5mL tubes (Thermo Scientific Cat #75004081 or similar)
3. Heat block (Eppendorf Thermomixer C Cat #2231000667 or similar)
4. DNA LoBind Microcentrifuge Tubes 1.5mL (Thermo Scientific, Cat #13-698-791)

Reagents

1. Allprep RNeasy Mini kit (Qiagen, Cat #80204)
 - a. AllPrep DNA Mini Spin Columns in 2mL collection tube
 - b. RNeasy Mini Spin Columns in 2mL collection tube
 - c. Buffer RLT Plus
 - d. Buffer RW1
 - e. Buffer RPE
 - f. RNase-Free Water
 - g. Buffer AW1
 - h. Buffer AW2
 - i. Buffer EB
2. RNase Free DNase set (Qiagen, Cat # 79254 or Cat #79256)
 - a. RNase-free DNase I
 - b. RNase-free Buffer RDD
 - c. RNase-free water
3. β -Mercaptoethanol (Thermo Fisher Scientific, Cat #35602BID)
4. Mol. grade isopropanol, 100% (Fisher Scientific, Cat #BP2618500 or similar)
5. Non-denatured, mol. grade ethanol, 200 proof (Fisher Scientific, Cat #BP2818100)
6. RNase/DNase free water (Thermo Fisher Scientific, Cat #10977015 or similar)
7. RNaseZap Decontamination Solution (Thermo Fisher Scientific, Cat #AM9782 or similar)

General Practices and Notes

1. Pre-chill microcentrifuge to 4°C.
2. Turn the heat block on and set it to 70°C and heat 2mL of RNase/DNase Free Water.
3. Prepare lysis buffer master mix by adding 1% β -ME to RLT buffer (10uL β -ME in to 1mL of RLT buffer).
 - a. If using homogenization method described in SOP #2.1 prepare 600uL-800uL lysis buffer per sample.
 - b. If using homogenization method described in SOP #2.2 prepare 1mL lysis buffer per sample.

4. **Make sure 100% EtOH is added to all the Qiagen concentrated buffers RPE and RW1.**
5. Set a microcentrifuge to room temperature (25°C).
6. Prepare a working rack with pre labeled tubes as illustrated in **Figure 1**.

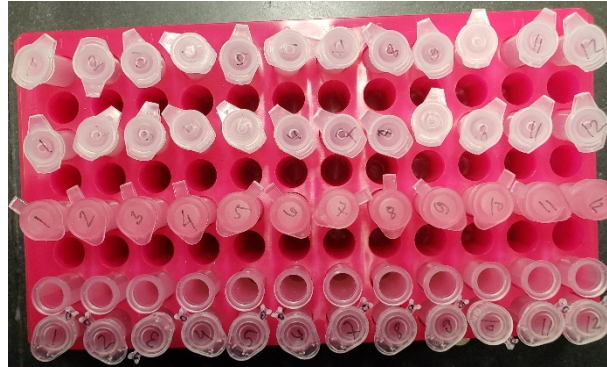


Figure 1: Tube layout on a tube rack.

Procedure

1. Follow tissue homogenization SOP #2.1 or SOP#2.2. After completing the homogenization SOP, you'll have a 1.5mL tube with lysate.
2. Centrifuge the lysate in the 1.5mL tubes for 3min at full speed in the room temp centrifuge.
3. Transfer the supernatant avoiding the pellet to the AllPrep DNA Mini Spin Column in 2mL collection tube. Centrifuge for 30 sec at 9,400 RCF and RT.
4. Place the AllPrep DNA Mini Spin Column in a new 2ml collection tube. Store at 4°C (or on wet ice) for later DNA purification.
5. Use the flow through for RNA purification below following steps 6-18 below:
6. Add 1 volume of freshly prepared 70% ethanol to the flow through from step 5, (adjust based on the starting RLT buffer volume). Mix well by pipetting and do not centrifuge. Proceed immediately to step 7.
 - a. For maximum RNA yields from liver, 50% ethanol (instead of 70% ethanol) should be used. Based on AllPrep DNA/RNA Mini-Handbook pg26.
7. Transfer up to 700µL sample to the RNeasy spin column (pink) placed in a 2ml collection tube. Centrifuge for 30 sec at 10,000 RCF and RT. Discard the flow-through, transfer remainder sample to spin column.
 - a. Repeat step until all lysate has passed through the column.
8. Add 350µL Buffer RW1 to the RNeasy spin column, and centrifuge for 30 sec at 10,000 RCF and RT. Discard the flow-through.
9. Prepare DNase stock solution (if no current stock is available) and DNase master mix.



- a. Dissolve the solid DNase I (1500 Kunitz units) in 550 μ L of the RNase-free water provided. Take care not to lose any of the solid DNase I when opening the vial.
 - b. For long-term storage of DNase I, transfer the stock solution from the glass vial, aliquot in several tubes, and store at -20°C for up to 9 months. Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not re-freeze the aliquots after thawing.
 - c. To make the master mix, for each sample combine 10 μ L DNase and 70 μ L RDD buffer.
- Never vortex reconstituted DNase I or the master mix!***
10. Pipette 80 μ L DNase I master mix directly onto RNeasy Mini column membrane of each sample and incubate at room temperature for 30min.
11. Add 350 μ L Buffer RW1 to the RNeasy spin column, and centrifuge for 30sec at 10,000 RCF and RT. Discard the flow-through.
12. Add 500 μ L Buffer RPE to the RNeasy spin column, and centrifuge for 30sec at 10,000 RCF and RT. Discard the flow-through.
13. Repeat step 12.
14. Transfer the column in to a new 2ml collection tube and centrifuge at full speed for 2min and RT to dry the RNeasy silica-gel membrane. Discard the collection tube when done.
15. Transfer the column to 1.5ml collection tube and air dry with open caps for 10min.
16. Add 50 μ L RNase-free water directly onto RNeasy silica-gel membrane. Incubate at RT for 5-10min.
17. Close tube gently, and centrifuge at 8,000 RCF for 1min at RT.
 - a. If the volume recovered is lower than the anticipated amount, perform additional spin of 1min at full speed.
 - b. Second elution of 30 μ L RNase-free water can be performed to get higher yield.
18. Follow SOP #4.1 perform RNA quantification using Qubit fluorimeter.
19. Follow SOP #4.3 perform RNA quality analysis using Bioanalyzer.
20. Aliquot the generated samples following SOP #1.1
21. Add 500 μ L Buffer AW1 to the AllPrep DNA spin column from pre-processing step 4. Centrifuge for 30sec at 10,000 RCF and RT. Discard the flow-through.
22. Add 500 μ L Buffer AW2 to the AllPrep DNA spin column. Centrifuge for 2min at full speed to wash and dry the spin column membrane.
23. Place the AllPrep DNA spin column in a new 1.5mL collection tube (supplied with the AllPrep Tube). Add 50 μ L warm (70°C) RNase/DNase Free water directly to the spin column membrane. Incubate at RT for 2min, and then centrifuge for 1min at 8,000 RCF at RT to elute the DNA.
 - a. A second elution of 30 μ L can be performed to get higher yield.
24. Following SOP #4.1 perform DNA quantification using Qubit fluorimeter.
25. Following SOP #4.2 perform DNA quality analysis using TapeStation.
26. Aliquot the generated samples following SOP #1.1

Appendix A—Protocol adjustments for mouse muscle tissue

RNA yields from skeletal muscle, heart and skin tissue may be low due to the abundance of contractile proteins, connective tissue and collagen. There are couple of protocol adjustments to assure sufficient recovery. The below procedure outlines steps from this SOP that can be adjusted:

1. Starting material: no more than 15mG of LN2 preserved tissue. 10mG RNAlater preserved tissues.

Table 1: Table shows the protocol adjustments needed to yield enough high-quality RNA.

Step	Adjustment
Prep	RNA: Warm an aliquot of RPE and RW1 buffers to 37°C for 30min: each sample requires 1.2uL of RW1 and 2ml of RPE. DNA: Is usually not a problem, follow similar procedural adjustments if the column appears to be clogged after the lysate pass through.
Step 2	Repeat the step for a total of 2 times to avoid as much cell debris as possible
Step 7	Load up to 400uL of lysate on to the column. Repeat until all lysate passed through the column
Step 8	Use 350uL pre-warmed RW1 buffer
Step 11	Use 700uL pre-warmed RW1 buffer. RW1 buffer washes out organic contaminants like proteins that are abundant in muscle/skin tissues thus the volume is increased
Step 12-13	Use 500uL of pre-warmed RPE buffer
Step 15	Elute twice in 50ul and 30ul of 37°C RNase-free water, respectively. Measure the concentration and combine if the second elution is 30% of the first or higher.
Troubleshooting	If at any point the column clogged, repeat the wash step by loading the wash solution on to the column and incubating sample at 37°C for 5 min prior the centrifugation.
Troubleshooting	If the above adjustments did not improve the yield: for each sample homogenize 2X15mg aliquots and process separately until step 15. Combine the elutants for total yield evaluation.



2. Similar adjustment can be used for skin tissue but using 37°C buffers might result in lower quality of RNA. Muscle tissues tend to be robust and yield good quality RNA (RIN 8-10) whereas skin on the other hand tends to be high in RNases and typically, yields lower quality RNA (RIN 4-6), so this protocol is not recommended. Use SOP #3.2 for RNA extraction from skin.