QIAGEN Allprep mini RNA extraction with QIAGEN RNase-Free DNase Set.

vggh

Abstract

Validated with : rodent liver, kidney, eye, skeletal muscles, adrenal gland.

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Guidelines

1. Make sure to seperate RNA and DNA work
2. Work quickly and efficiantly to preved RNA degradation
3. Make sure to not thaw the tissues at the time of weighting, work on dry ice.
4. Stopping point at DNase digestion step
5. At the time of the homogenization, keep the samples on wet ice

Before start

1. Prepare the lysis buffer on the day of the extraction by adding 1% of beta-mercaptoethanol to the total needed RLT volume.
2. Use 1000ul of RLT+1% β-ME per sample if starting with the suggested amount of 20+/-5mg of tissue (Muscles<20mg, all other tissues </=30mg)
3. Confirm that ethanol was added to buffer RPE, AW1, AW2
4. Prepare the DNase right before the digestion step.
5. Prepare 2 buckets of wet ice : for RNA samples post trap-on-column step and for tissue lysate and DNA samples.

Materials

Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich

RNeasy Mini Kit 74104 by Qiagen

DNase I, RNase free EN0525 by Thermo Fisher Scientific

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Protocol

Step 1.

Homogenize the sample in up to 1ml of RLT buffer(+β-ME) using Polytron large probe at 20K rpm, make sure to break every 15 seconds to allow the probe to cool off . For non muscle tissue - 2 repeats, for muscles - 3 homogenization rounds or untill homogenious. Take care to avoid foaming.

Step 2.

Centrifuge the lysate for 3 minutes at full speed (RT). Transfer up to 600ul of the supernatant, avoiding the pelleted cell debris in to an Allprep DNA mini spin column.

Step 3.

Centrifuge DNA spin column for 30 sec at 10,000 rpm

Step 4.

Repeat untill all supernatant passed the DNA column.

NOTES
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Make sure to keep the flow through that contains RNA.

Step 5.

Place the Allprep DNA spin column in a new 2ml collection tube (supplied). And store at 4°C for later DNA purification. Use the flow through for RNA purification below.

Step 6.

Add 713 µl of 100% ethanol to the flow through, (adjust based on starting RTL volume). Mix well by pipetting or vortexing all of the tubes at once. Proceed immediately to step 5. Adjust volume of ethanol based on the starting volume of RLT buffer.

Step 7.

Transfer up to 600 µl sample to the RNeasy mini spin column. Centrifuge for 15 sec at 10,000 rpm. Discard the flow-through. Repeat step 5 until all the lysate+ethanol had passed through the column.

Step 8.

Add 350 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 sec at 10,000 rpm to wash the spin column membrane. Discard the flow-through.

Step 9.

Prepare the DNase master mix by combining 10ul DNase with 70ul RDD buffer per each sample.

Step 10.

Add 80 µl of DNase master mix to each RNeasy spin column membrane, and incubate on the bench top for at least 30 min. (No longer than 1 hour)

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Step 11.

Add 350 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 sec at10,000 rpm to wash the spin column membrane. Discard the flow-through.

Step 12.

Add 500 µl Buffer RPE to the RNeasy spin column, and centrifuge for 15 sec at 10,000 rpm to wash the spin column membrane. Discard the flow-through. Repeat step.

Step 13.

Add 500 µl Buffer RPE to the RNeasy spin column, and centrifuge for 2 min at 10,000 rpm to wash the spin column membrane. Discard the flow-through.

Step 14.
Air dry samples for 10 min, then place the RNeasy spin column in a new 2 ml collection tube, and centrifuge at full speed for 1 min.

Step 15.

Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 50 µl RNase-free water directly to the spin column membrane. Incubate at RT for 2 minutes before centrifuging for 1 min at 10,000 rpm to elute the RNA. A second elution of 30 µl RNase-free water can be performed to ensure complete yield.

Step 16.
Quantify concentration by Qubit RNA BR.

EXPECTED RESULTS

Total amount needed for sequencing is 2ug

Step 17.
Measure RNA quality by Bioanalyzer Nanochip or Picochip.

EXPECTED RESULTS

RIN of above 7 for uncompromised mammalian tissues

Step 18.

Perofm ERCC spike in addition for sequencing designated aliquote

Warnings

- When working with QIAGEN column extraction kits, avoid using bleach.

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