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We use this protocol and it's working

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RNA Extraction and RIN assessment.

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DISCLAIMER

Protocol Adapted from Mauricio Rodriguez-Lanetty and Qiagen's RNA Pico 6000 user manual

ABSTRACT

Adapted from Mauricio Rodriguez-Lanetty

GUIDELINES

Important:
Work in the fume hood in steps 1-7

- Do not worry about RNases in steps 1-5. Your sample is full of them anyway. They are inhibited as long as they are in Trizol.
- From step 6 onwards you should be careful of not contaminating the samples with RNases. Keep cleaning your gloves with RNase Zap (Ambion) through the whole process. The main source of contamination comes from your fingers by accidentally touching the inner part of the tube caps.
- While discarding flow-through in steps 8-12, avoid touching the mouth of the collection tubes with anything!

MATERIALS

RNeasy Mini Kit **Qiagen Catalog #74104**

Chloroform **Fisher Scientific Catalog #BP1145-1**

TRIzol Reagent **Thermo Fisher Scientific Catalog #15596026**

Equipment

	NAME
Bioanalyzer 2100 instrument	BRAND
G2939BA	SKU
with RNA 6,000 Pico LabChip kit	SPECIFICATIONS

Equipment

TissueRuptor II	NAME
Qiagen	BRAND
9002755	SKU

RNA Extraction

36m

1 Homogenize the starting material using a TissueRuptor and the appropriate volume of Trizol (see table, check Trizol instructions).

	A	B
	Amount of Tissue	Trizol Volume
	100mg	1ml
	50mg	0.5ml

2 Let the homogenate sit at room temperature for 5 min. 00:05:00 5m

3 Optional: Centrifuge for 10 min at 12,000xg at 4C to eliminate debris and polysaccharides. 10m
12000 rcf, 4°C, 00:10:00 . Collect the supernatant.

4 Add chloroform to the homogenate (0.2 ml chloroform per ml Trizol used) and shake vigorously for 20 sec, 3m
then allow the sample to sit at room temperature for 2-3 min. 00:03:00

	A	B
	Trizol used in step 1	Chloroform
	1ml	200ul
	0.5ml	100ul

5 Spin at 10,000g at 4C for 18mins. 10000 rcf, 4°C, 00:18:00 18m

6 Carefully remove aqueous phase (top) by aspiration (use sterile disposable fine plastic pipette) and transfe 1m
to a new sterile RNase-free tube (1.5 ml tube).
IMPORTANT: Stay away from the aqueous/organic interphase. This is where the DNA and RNases are. It is suggested to sacrifice aqueous material rather than risking taking this precipitate.

7 Slowly add an equal volume of 100% RNAse-free EtOH, mixing it as needed. 5m

8 Load the sample (up to 700µl) into an RNeasy column (Qiagen kit) seated in a collection tube and spin for 30 sec at 8,000g. Discard flow-through. 30s

8000 rcf, Room temperature, 00:00:30

9 If planning to run the samples on BioAnalyzer right away, move RNApico reagents to room temperature, protecting them from light (~30 mins before use)

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10 Add 700µl buffer RW1 onto column and spin 30 sec at 8,000g. Discard flow-through. 30s

8000 rcf, Room temperature, 00:00:30

11 Transfer column into a new 2ml collection tube, add 500µl buffer RPE and spin for 30 sec at 8,000xg. Discard flow-through. **Make sure ethanol has been added to the RPE buffer before use**. 30s

8000 rcf, Room temperature, 00:00:30

12 Add 500µl buffer RPE and spin 2 min at 8,000xg. Discard flow-through. 2m

8000 rcf, Room temperature, 00:02:00

13 Spin the column for 1 min at 8,000xg to get rid of the remaining buffer in the column. 1m

8000 rcf, Room temperature, 00:01:00

14 Transfer the column to a new 1.5ml collection tube and pipet 30-50µl of RNase-free water directly onto column membrane. Allow the sample to sit at room temperature for 1-2 min 00:01:30 , and then spin 1 min at 8,000xg to elute RNA. 8000 rcf, Room temperature, 00:01:00 2m 30s

15 Store RNA at -80C if not using it immediately.

Analysis on BioAnalyzer

27m

16 Preparing the Gel. Previously prepared gels can last up to a month in the fridge.

16.1 Place 550 µl of RNA 6000 Pico gel matrix (red) into the top receptacle of a spin filter.

16.2 Place the spin filter in a microcentrifuge and spin for 10 minutes at 1500 g

10m

1500 rcf, 00:10:00

16.3 Aliquot 65 µl filtered gel into 0.5 ml RNase-free microcentrifuge tubes that are included in the ki

5m

Store the aliquots at 4 °C and use them within one month of preparation.

17 Prepare Gel-Dye Mix

17.1 Vortex RNA 6000 Pico dye concentrate (blue) for 10 seconds and spin down.

17.2 Add 1 µl of RNA 6000 Pico dye concentrate (blue) to a 65 µl aliquot of filtered gel (prepared as described in “Preparing the Gel” on Step 16).

17.3 Cap the tube, vortex thoroughly and visually inspect proper mixing of gel and dye. Store the dye concentrate at 4 °C in the dark again.

17.4 Spin tube for 10 minutes at room temperature at 13000 g 10m
13000 rcf, Room temperature, 00:10:00 . Use prepared gel-dye mix within one day.

18 Setting up the Chip Priming Station: 1m
- Base plate should be in **position C**.
- Adjust the syringe clip to **the top position**

19 Clean the Electrodes

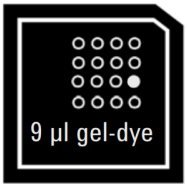
19.1 Slowly fill one of the wells of the electrode cleaner with 400 µl of fresh RNase-free water.

19.2 Open the lid and place the electrode cleaner in the Agilent 2100 Bioanalyzer instrument. Close the lid and leave it closed for 5 minutes. 5m
00:05:00

19.3 Open the lid and remove the electrode cleaner. Wait another 30 seconds to allow the water on the electrodes to evaporate before closing the lid 30s
00:00:30

20 Loading the Gel-Dye Mix

20.1 Place the chip on the chip priming station. Pipette 9.0 µl of the gel-dye mix at the bottom of the well marked and dispense the gel-dye mix.



20.2 Set the timer to 30 seconds, make sure that the plunger is positioned at 1 ml and then close the chip priming station. The lock of the latch will click when the chip priming station is closed correctly.

20.3 Press the plunger of the syringe down until it is held by the clip. Wait for exactly 30 seconds an 30s then release the plunger with the clip release mechanism. 00:00:30

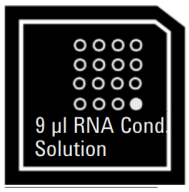
20.4 Visually inspect that the plunger moves back at least to the 0.3 ml mark. Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.

20.5 Open the chip priming station and pipette 9.0 µl of the gel-dye mix in each of the wells marked **G**

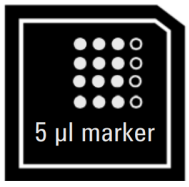
NOTE: discard the vial with remaining gel-dye mix.

21 Loading the RNA 6000 Pico Conditioning Solution and Marker

21.1 Pipette 9 µl of the RNA 6000 Pico conditioning solution (white cap) into the well marked CS.



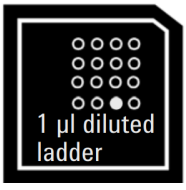
21.2 Pipette 5 µL of the RNA 6000 Pico marker (green cap) into the well marked with a ladder symbol and each of the 11 sample wells



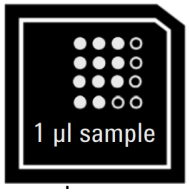
22 Loading the Diluted Ladder and Samples

22.1 Before use, thaw ladder aliquots and keep them on ice (avoid extensive warming upon thawing process)

22.2 Pipette 1 µl of the diluted RNA 6000 Pico ladder into the well marked with the ladder symbol



22.3 Pipette 1 µl of each sample into each of the 11 sample wells. Add 1 µL of deionized water to each unused sample well. **Do not leave any wells empty or the chip will not run properly**.



22.4 Place the chip horizontally in the adapter of the IKA vortex mixer and make sure not to damage the bulge that fixes the chip during vortexing. If there is liquid spill at the top of the chip, carefully remove it with a tissue.

22.5 Vortex for 60 seconds at 2400 rpm. Make sure that the run is started within 5 minutes.

23 Insert chip on BioAnalyzer and select Total RNA Pico as the assay. Select only the wells with Samples to save time.

24 Remove the chip immediately after the run. Clean electrodes with RNAse-free water.