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RNA Isolation from QIAzol sample with miRNeasy Mini Kit

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We use this protocol and it's working

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Abstract

Purpose

This **SOP** describes the **procedure** of **total RNAextraction** from **cell** and **tissue** **samples** using the Qiagen miRNeasy Mini kit and Ambion Turbo DNA-free kit. The miRNeasy Mini Kit combines phenol/guanidine-based **lysis** of samples and silica–membrane-based purification of total RNA. QIAzol Lysis Reagent, included in the kit, is a monophasic solution of phenol and guanidine thiocyanate designed to facilitate the **lysis of tissues**, **inhibit** RNases, and **remove** most of the cellular **DNA** and **proteins** from the lysate by **organic extraction**. **Cells** or **tissue** samples are **homogenized** in the QIAzol Lysis reagent. After adding chloroform, centrifugation separates the homogenate into aqueous and organic phases. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane, and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water. Eluted RNA is treated with an Ambion TURBO DNA-free kit to **remove contaminating DNA**. A final **cleanup** step by **Ethanol** precipitation removes traces of DNase **buffer** and **enzyme** to avoid effects on downstream applications, like **reverse transcription** of **RNA** into **cDNA**.

This **protocol** **extracts** the following classes of RNA: **total RNA** (including **mRNA**, **miRNA**, **rRNA**, **tRNA**, **snRNA**, **snoRNA**, **piRNA** and **lncRNA**)

Attachments

SOP_6_v4_RNA-Isolati...
227KB

LabTrackingForm_SOP-..
70KB

Guidelines

For Section-A: Steps are performed under the fume hood
For Section-B: Steps are performed under the PCR workbench
For Section-C: Steps are performed under the PCR workbench
For Section-D: Steps are performed under the PCR workbench

Materials

Required material:

- Chloroform (Th. Geyer, 2475.1000)
- 100% Ethanol (AppliCem, A3678-1L)
- 70% Ethanol (prepared from 100% Ethanol)
- RNase-free-Water (AppliChem, A7398,1000)
- RNaseAWAY (MBP, 7002)
- miRNeasy Mini Kit, including QIAzol, buffers, and spin columns (Qiagen, 217004)
- Maxtract High Density 2 ml tubes (Qiagen, 129056)
- Turbo DNA-free Kit (Ambion, AM1907)
- Glycogen – 5 mg/ml (Ambion, AM9510)
- 3M Sodium acetate buffer – pH 5,2 (Sigma-Aldrich, S7899-100ML)
- 1.5 mL SafeLockTubes – PCR clean (Eppendorf, 0030 123.328)
- 0.5 mL SafeLockTubes – PCR clean (Eppendorf, 0030 123.301)

Required equipment:

- Fume hood
- PCR workbench
- Eppendorf Centrifuge 5418R
- Eppendorf ThermoMixer C with ThermoTop
- Vortex-Genie 2 Digital
- -20°C freezer
- -80°C freezer
- Timer

Safety warnings

Wear gloves and a lab coat, and use eye protection. Ensure that any residual liquid nitrogen or dry ice has evaporated from the sample before closing the tube or adding solutions.

Before start

Each surface must be decontaminated with RNase AWAY beforehand. All pipetting steps must be performed under RNase-free conditions.

A. Prepare sample and material (starting from 1 mL QIAzol sample)

1 Place the tube containing the sample (cell pellets or homogenized tissue in QIAzol reagent) on the bench top at room temperature (15-25 °C) for 00:05:00 . 5m

2 At this time, centrifuge the MaXtract tube at 14.000 x g for 00:00:30 . 30s

3 Pre-cool the centrifuge to 4 °C .

4 Mix QIAzol-sample by pipetting or choose another appropriate homogenization method.

5 Transfer by pipetting 1000 µL QIAzol-sample to the MaXtract® tube.

6 Add 200 µL chloroform.

7 Securely cap the tube and shake it vigorously for 00:00:15 . DO NOT VORTEX! 15s

8 Place the tube on the bench top at Room temperature for 00:03:00 . 3m

9 Centrifuge at 12.000 x g, 4°C for 00:15:00 . 15m

10 In the meantime, label spin columns and 1.5 mL SafeLockTubes for ethanol mixture and elution (if necessary, prepare Buffer RWT and Buffer RPE as indicated in the protocol sheet “miRNeasy Mini”).

11 Pre-warm the centrifuge to 20 °C .

12 Transfer the upper, aqueous phase from the Maxtract tube to a new 1.5 mL safe-lock tube (by decanting).

13 Add 750 µL (around 1.5 volumes) of [M] 100 % (v/v) ethanol and mix thoroughly by pipetting.

14 Pipet 700 µL sample, including any precipitate, into an RNeasy Mini spin column in a 2 mL collection tube. Close the lid and centrifuge at room temperature at 8.000 x g for 00:00:30. Discard the flow-through.

30s

15 Repeat the last step (A14) using the remainder of the sample.

B. Wash spin column and elute RNA

16 Add 700 µL Buffer RWT to the spin column. Close the lid and centrifuge for 00:00:30 at 8.000 x g. Discard the flow-through.

30s

17 Add 500 µL Buffer RPE to the spin column. Close the lid and centrifuge for 00:00:30 at 8.000 x g. Discard the flow-through.

30s

18 Add another 500 µL Buffer RPE to the spin column. Close the lid and centrifuge for 00:02:00 at 8.000 x g. Discard the flow-through.

2m

19 Place the spin column into a new 2 ml collection tube and centrifuge at 12.000 x g for 00:01:00 to dry the membrane further.

1m

20 Transfer the spin column to a new 1.5 mL SafeLockTube. Pipet 30 µL RNase-free water directly onto the spin column membrane. Close the lid and centrifuge for 00:01:00 at 8.000 x g to elute.

1m

21 Remove and discard spin columns from elution tubes.

22 Put elution tubes with total RNA On ice .

C. DNase digestion

23 Preheat the ThermoMixer with 0.5 mL block and ThermoTop at 37 °C .

24 Prepare and label 0.5 mL SafeLockTubes.

25 Add 3 µL 10x Turbo DNase Buffer to the samples.

26 Add 1.5 µL Turbo DNase to the samples.

27 Transfer samples to 0.5 mL SafeLockTubes and mix gently by pipetting.

28 Incubate samples for 00:30:00 at 37 °C . 30m

29 Take samples from the ThermoMixer and add another 1.5 µL Turbo DNase. Mix by pipetting.

30 Incubate the samples for 00:30:00 at 37 °C 30m

31 During this time, prepare and label 1.5 mL SafeLockTubes for digested total-RNA and thaw DNase Inactivation reagent.

32 Remove the samples from the ThermoMixer and add 7.2 µL of well-mixed Turbo DNase Inactivation reagent. Mix by pipetting.

33 Incubate for 00:05:00 at room temperature, mixing occasionally by vortexing at 1800 rpm . 5m

34 Centrifuge at 10.000 x g for 00:01:30 . 1m 30s

35 Transfer as much RNA as possible (upper, aqueous phase) to 1.5 mL SafeLockTubes by pipetting. Don't transfer some of the white sediment to the new tube.

D. RNA precipitation

36 Add 140 µL RNase-free water to the sample.

37 Add 4 µL Glycogen to the samples.

38 Add 20 µL 3M Sodium Acetate to the samples and mix by pipetting.

39 Add 600 µL of ice-cold 100% ethanol to each sample and mix by pipetting.

40 Place samples at -20 °C Overnight . 5m

41 The next day: Pre-cool the centrifuge to 4 °C .

42 Centrifuge samples at 12.000 x g, 4°C for 00:30:00 . 30m

43 Carefully remove and discard the supernatant.

44 Wash the pellet with 500 µL ice-cold [M] 70 % (v/v) ethanol and vortex the solution briefly.

45 Centrifuge samples at 7.600 x g, 4°C for 00:05:00 . 5m

46 Carefully remove and discard the supernatant.

47 Wash the pellet with another 500 µL ice-cold [M] 70 % (v/v) ethanol and vortex the solution briefly.

48 Centrifuge samples at 7.600 x g, 4°C for 00:05:00 . 5m

49 Carefully remove and discard the supernatant.

50 Centrifuge samples at 7.600 x g, 4°C for 00:00:30 . 30s

51 Carefully remove and discard any rest of the ethanol by pipetting.

52 Under the PCR workbench, with the tube lid open, allow the pellet to air dry at room temperature (do not overdry the pellet).

53 At this time, preheat the ThermoMixer with 1.5 mL block and ThermoTop at 58 °C .

54 Dissolve the pellet in 20 µL of RNase-free water by pipetting up and down.

55 Incubate samples for 00:08:00 at 58 °C in a ThermoMixer. 8m

56 Place the samples On ice to cool down. Mix the samples by flicking. Centrifuge briefly to collect the entire sample.

57 Store total RNA at -80 °C .

E. Cleaning

58 Clean all used materials and put them back.

59 Empty all waste bins and discard collection of flow through.

60 Clean all used working areas with RNaseAWAY.

61 Turn off all used instruments.

62 Turn on the PCR workbench UV light for 30 minutes.

Protocol references

Reference documents (basis for this protocol):

- Qiagen miRNeasy Mini Handbook 11/2020 (https://www.qiagen.com/us/resources/resourcedetail?id=da6c8d17-58c4-411c-a334-bc1754876db3&lang=en)

- Ambion Turbo DNA-free Manual (https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2F1907M_turbodnafree_UG.pdf)