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We use this protocol and it's working

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Purification of Total RNA from Cells Using Spin Technology

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ABSTRACT

Purification of Total RNA from Cells Using Spin Technology

MATERIALS

Equipment:
Sterile,
RNase-free pipet tips
Microcentrifuge
Disposable gloves
TissueLyser
QIAshredder (cat#79656 Qiagen)

Reagent:
RNeasy mini kit
(cat#74106 Qiagen)
RNase
ZAP(cat#AM9780 Ambion)
β-mercaptoethanol
96–100%
ethanol
PBS
0.25% trypsin

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Method

1 Add 20µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.

2 Add volumes of ethanol (96–100%) as indicated on the bottle to concentrate buffer RPE to obtain a working Solution.

3 Harvest cells (do not use more than 1 x 107 cells) To lyse cells directly in the cell-culture vessel (up to 10am diameter): Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 4. To trypsinize and collect cells (Cells grown in cell-culture flasks should always be trypsinized): Determine the number of cells. Detach cells from the dish or flask using trypsin, add medium containing serum to inactivate the trypsin, transfer the cells to an RNase-free centrifuge tube, and centrifuge at 1200rpm for 5 min. Completely aspirate the supernatant, and proceed to step 4.

4 Disrupt the cells by adding Buffer RLT. For direct lysis of cells: Add the appropriate volume of Buffer RLT as below to the cell-culture dish. Collect the lysate with cell scrapter. Pipet the lysate into a microcentrifuge tube. Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 5.

	A	B
	Dish diameter (cm)	Volume of Buffer RLT (µl)
	<6	350
	6-10	600

For pelleted cells: Loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of Buffer RLT as below. Pipet to mix, and proceed to step 5.

	A	B
	Number of pelleted cells	Volume of Buffer RLT (µl)
	<5 x 10^6	350
	5x 10^6 – 1 x 10^7	600

5 Homogenize the lysate. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed.

6 Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting.

7 Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at 10,000 rpm. Discard the flow-through *If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.*

8 Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 10,000 rpm to wash the spin column membrane. Discard the flow-through

9 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 10,000 rpm to wash the spin column membrane. Discard the flow-through.

10 Repeat step7.

11 Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at 10,000 rpm to elute the RNA.

Note:

12 Always wear gloves and spray with RNaseZap and spread solution all over gloves

13 Cell pellets can be stored at –70°C for later use or used directly in the procedure. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube.

14 Homogenized cell lysates from step 5 can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x g. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 6.

15 Perform all steps of the procedure at room temperature. During the procedure, work quickly.

16 Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C