Isolation of total RNA from adherent cells with RNeasy kit (Qiagen)

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We use this protocol since years for different types of mammalian cells. The cells should be grown in a 90mm Petri dish to monolayer. Please note, that this protocol does not substitute the handbook provided by Qiagen and was thought to provide a quick reference.

Important notes before you start

- Always wear gloves while working with RNA.
- Change your gloves as often as you can.
- Clean your bench with RNase-Erase or similar RNase killer.
- Use filtered pipet tips if possible.

Protocol

1. Prepare the lysis buffer (700 µl per 90 mm dish) by adding 10 µl of β-mercaptoethanol to each 1 ml of the RLT buffer. Mix well.
2. Aspirate culture medium from the Petri dish.
3. Hold the dish inclined for 30 sec, aspirate the rests of the culture medium.
4. Pipet 600µl of the lysis buffer onto the dish, distribute the buffer evenly by rocking the dish.
5. Using the cell scraper collect all the lysis buffer on one side of the dish.
6. Transfer the lysate into a 2 ml tube.
7. Homogenize the lysate by passing it 10 times through a 20G needle.
8. To homogenized lysate add 1 volume (600 µl) of 70% ethanol.
9. Vortex thoroughly.
10. Transfer 700 µl of the sample to a RNeasy column, placed in provided 2 ml tube.
11. Centrifuge 1 min at 13000 rpm.
12. Discard flow-through.
13. Repeat step 10 by transferring the rest of sample to the column.
14. Centrifuge 1 min at 13000 rpm.
15. Discard flow-through.
16. Apply 600 µl of RW1 buffer to the column.
17. Centrifuge 1 min at 13000 rpm.
18. Discard flow-through.
19. Apply 500 µl of buffer RPE to the column.
20. Centrifuge 1 min at 13000 rpm.
21. Place RNeasy column in a fresh 2 ml tube (provided).
22. Apply 500 µl of buffer RPE to the column.
23. Centrifuge 3 min at 13000 rpm.
24. Place RNeasy column in a collection tube.
25. Apply 50 µl of hot (60-70°C) RNase free water directly to the membrane.
26. Incubate 1 min.
27. Centrifuge 1 min at 13000 rpm.
28. Apply another 50 µl of hot (60-70°C) RNase free water to the membrane.
29. Centrifuge 3 min at 13000 rpm.
30. Transfer eluate into a fresh RNase free tube (not provided), determine the concentration photometrically.
31. The quality of the obtained RNA samples may be analyzed on a standard 1% agarose, 1x TAE gel (figure 1).
32. Store isolated samples at –80°C.

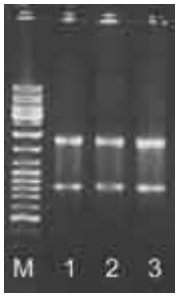

Figure 1. M – DNA marker
1, 2, 3 – RNA samples