RNA isolated methods

Step-by-step protocols

Qiagen miRNeasy kit (RN)

1. Add 1400 µl QIAzol Lysis Reagent to 200 µl sample in a 2 ml tube and disrupt and homogenize sample by shaking for 30''.
2. Place tube containing homogenate at room temperature for 5 min.
3. Add 20 µl 10 pM cel-miR- 39
4. Add 280 µl chloroform and cap tube securely. Shake vigorously for 15 sec.
5. Place tube at room temperature for 2-3 min.
6. Centrifuge for 15 min. at 12,000 x g at 4°C.
7. Transfer upper aqueous phase (800 µl) to new 2 ml collection tube. Avoid pipetting the (white) interface. Add 1.5 volumes of 100% ethanol (i.e. 1.2 ml) and mix thoroughly by pipetting.
8. Pipet up 650 µl of sample, including any precipitate, onto an RNeasy Mini column in 2 ml collection tube. Close lid, centrifuge at 10.000 rpm for 15 sec. at room temperature (15-25°C). Discard flow-through.
9. Repeat step 8 until all of the sample has been loaded on the cloumn.
10. Add 700 µl Buffer RWT to the RNeasy Mini column. Close lid, centrifuge for 15 sec. at 10.000 rpm. Discard flow-through.
11. Pipet 500 µl Buffer RPE onto RNeasy Mini column. Close lid, centrifuge for 15 sec. at 10.000 rpm. Discard flow-through.
12. Add 500 µl Buffer RPE to RNeasy mini column. Close lid, centrifuge for 2 min. at 10.000 rpm.
13. Dry the outside of the column with a clean tissue.
14. Transfer RNeasy Mini column to new 1.5 ml collection tube from the box. Pipet 30 µl RNase-free water directly onto RNeasy Mini column membrane. Close lid, centrifuge for 1 min. at 10.000 rpm to elute.
15. Re-apply the 30 µl eluate to the column and spin again to completely elute
16. Store at -80°C

Qiazol and Dr. Gentle Total RNA isolation (QP)

1. Add 1 ml QIAzol Lysis Reagent to 100 µl sample in a 2 ml tube and disrupt and homogenize sample by shaking for 30'. Do this in duplicate! (i.e. You have 2 tubes each with 100 µl per sample)
2. Place tubes containing homogenate at room temperature for 5 min.
3. Meanwhile, add 10 µl 10 pM cel-miR-39 to each tube
4. Add 200 µl chloroform per ml Qiazol used and cap tube securely. Shake vigorously for 15 sec.
5. Place tube at room temperature for 2-3 min.
6. Centrifuge for 15 min. at 12,000 x g (=rcf) at 4°C.
7. Transfer upper aqueous phase of the same sample (2x 500 µl) to new 2 ml collection tube.

8. Add 100 µl 3M NaAc (pH 5.2) (i.e. 1/10 V of the total aqueous phase) and short vortex
9. Add 1 µl of Dr. Gentle precipitation solution per 100 µl upper phase (= 10 µl) and short vortex.
10. Add 1 ml (0.5 volume of iso-propanol per ml Qiazol used = 2 ml) and mix thoroughly by pipetting.
11. Incubate 10 min at RT.
12. Centrifuge for 10 min. at 12,000 x g at 4°C
13. Carefully remove the supernatant with a vacuum pump. Use RNAse free tips!
14. Wash the “pellet” with 1 ml of 75% ethanol. Vortex
15. Centrifuge 5’, 7500 x g at 4□C.
16. Remove the supernatant and repeat step 14-15
17. Dry for 10’ at RT
18. Dissolve in 30 µl RNAse free water
19. Store at -80°C

NORGEN total RNA purification kit (NG)

1. Add 10 µl β-mercapto-ethanol per ml RL buffer just prior to use
2. Add 0.7ml RL buffer (containing β-mercapto) to 0.2 ml sample in a 2 ml tube.
3. Vortex ~15 seconds
4. Add 20 µl 10 pM cel-miR-39
5. Add 0.4 ml 100% ethanol
6. Vortex ~10 seconds
7. Place each column in a collection tube without a lid
8. Load 650 µL sample onto the column in a collection tube
9. Centrifuge 1’ 8000 RPM at RT
10. Discard the flow-through
11. Repeat steps 8-10 until the complete sample is loaded
12. Add 400 µl Wash solution A
13. Centrifuge 1 min at 13200 RPM at RT and discard flow-through
14. Repeat steps 12-13 2x (=3 times washing in total)
15. Spin 2’ at 13200 RPM at RT to dry column
16. Dry the outside of the column with a clean tissue
17. Place the column in a fresh 1.5 ml tube
18. Add 50 µl Elution solution A
19. Spin 2 min at 2000 RPM directly followed by a…
20. Spin 1 min at 13200 RPM
21. Check eluted volume. If<50 µl then repeat step 18.
22. Store at -80°C

mirCURY RNA Isolation Kit - Biofluids (CU)

1. Add 60 µl Lysis solution to 0.2 ml sample.

2. Vortex ~5 seconds
3. Place tube containing homogenate at room temperature for 3 min.
4. add 20 µl of 10 pM cel-miR-39
5. Add 22 µl Protein precipitation solution
6. Vortex ~5 seconds
7. Place tube at room temperature for 1 min.
8. Centrifuge for 3 min. at 11000 x g at RT.
9. Transfer clear supernatant to a new 2 ml tube.
10. Add 270 µl isopropanol
11. Vortex ~5 seconds
12. Load sample onto the column in a fresh collection tube
13. Incubate 2' at RT
14. Centrifuge 30" 11000 x g at RT
15. Discard the flow-through
16. Add 100 µl Wash solution 1
17. Centrifuge 30" 11000 x g at RT and discard flow-through
18. Add 700 µl Wash solution 2
19. Centrifuge 30" 11000 x g at RT and discard flow-through
20. Add 250 µl Wash solution 2
21. Centrifuge 2 min, 11000 x g at RT
22. Place the column in a fresh 1.5 ml tube and add 25 µl RNAse-free water direct on the column
23. Incubate at RT for 1 min.
24. Centrifuge for 1 min. at 11000 x g at RT.
25. Repeat step 22-24
26. Store at -80°C