1. miRNA extraction from liquid samples (plasma/serum)

1. defrost the plasma/serum sample on ice (do not vortex, but invert to mix)
2. transfer 400 ul of plasma into fresh tube (2 ml)
3. add 1200 ul Tri-Reagent LS (Invitrogen) and vortex samples
4. incubate for 5 min at RT, while incubating:
    - add 2 ul cel-mir-39 (5 fmol/ul)
    - add 10 ug glycogen (20 mg/ml, RNA grade) and vortex
5. add 320 ul chloroform and vortex each sample immediately for 5-10 s
6. vortex all samples vigorously (count `till 50) and incubate 10 min at RT
7. centrifuge for 15 min at 12 000 g (8°C)
8. carefully transfer the supernatant (~800 ul) into a fresh tube (2 ml) (on ice!)
   (keep interphase and lower phase if DNA extraction is desired)
9. add 1,5 volumes ethanol and mix by pipetting up and down several times
10. add sample in 700 ul aliquots to mini spin column (Qiagen miRNeasy Kit) (centrifuge each time at ~10 000 g for 10 s and discard flow through)
11. wash the column once with 700 ul RWT Buffer
12. wash with 500 ul RPE Buffer and centrifuge at ~10 000 g for 15 s
13. wash with 500 ul RPE Buffer and centrifuge at ~10 000 g for 2 min
14. spin column dry for 1 min at max. speed
15. transfer column to a fresh collection tube (2 ml)
16. add 30 ul RNase-free-water and centrifuge ~10 000 g for 1 min
17. apply first eluate for a second time to the same column and centrifuge ~10 000 g for 1 min
18. store eluate at -80°C

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2. miRNA extraction from whole blood

1. defrost the whole blood sample on ice
2. transfer 200 µl of diluted whole blood* sample into fresh tube (2 ml)
3. add 750 µl TRI Reagent BD (supplemented with 20 µl of 5 N acetic acid) and vortex
4. incubate for 5 min at RT
5. add 200 µl chloroform and vortex each sample immediately for 5-10 s
6. vortex all samples vigorously and incubate 5 min at RT
7. centrifuge for 15 min at 12 000 g (8°C)
8. carefully transfer the supernatant (~450 ul) into a fresh tube (2 ml) (on ice!)

(keep interphase and lower phase if DNA extraction is desired)

9. add 1,5 volumes ethanol and mix by pipetting up and down several times
10. add sample in 700 µl aliquots to mini spin column (Qiagen miRNeasy Kit) (centrifuge each time at ~10 000 g for 10 s and discard flow through)
11. wash the column once with 700 µl RWT Buffer
12. wash with 500 µl RPE Buffer and centrifuge at ~10 000 g for 15 s
13. wash with 500 µl RPE Buffer and centrifuge at ~10 000 g for 2 min
14. spin column dry for 1 min at max. speed
15. transfer column to a fresh collection tube (2 ml)
16. add 30 µl RNase-free-water and centrifuge ~10 000 g for 1 min
17. apply first eluate for a second time to the column and centrifuge ~10 000 g for 1 min
18. store eluate at -80°C

* 100 µl whole blood + 100 µl PBS (without Mg/Ca)

3. miRNA extraction from cells/tissue

1. determine amount of starting material according to the Qiagen miRNeasy Kit manual
2. add 700 ul QIAzol Lysis Reagent and vortex samples
3. homogenize cells using a syringe and needle
4. incubate for 5 min at RT
5. add 140 µl chloroform and vortex each sample immediately for 5-10 s
6. vortex all samples vigorously and incubate 5 min at RT
7. centrifuge for 15 min at 12 000 g (8°C)
8. carefully transfer the supernatant (~350 ul) into a fresh tube (2 ml) (on ice!)
   (keep interphase and lower phase if DNA extraction is desired)
9. add 1,5 volumes ethanol and mix by pipetting up and down several times
10. add sample in 700 µl aliquots to mini spin column (Qiagen miRNeasy Kit) (centrifuge each time at ~10 000 g for 10 s and discard flow through)
11. wash the column once with 700 µl RWT Buffer
12. wash with 500 µl RPE Buffer and centrifuge at ~10 000 g for 15 s
13. wash with 500 µl RPE Buffer and centrifuge at ~10 000 g for 2 min
14. spin column dry for 1 min at max. speed
15. transfer column to a fresh collection tube (2 ml)
16. add 30 µl RNase-free-water and centrifuge ~10 000 g for 1 min
17. apply first eluate for a second time to the column and centrifuge ~10 000 g for 1 min
18. store eluate at -80°C