

## RNA Purification from Blood Plasma & Serum - Human

### Material

miRNeasy mini kit (50), Qiagen, cat no. 217004

Extra RPE-buffer, Qiagen, Cat. No. 1018013

MS2 RNA (0.8 µg/µl). Roche, cat. No. 10165948001

Chloroform. Sigma Aldrich, Cat. No. C2432 (4x25 ml)

Abs. Ethanol (99.9%)

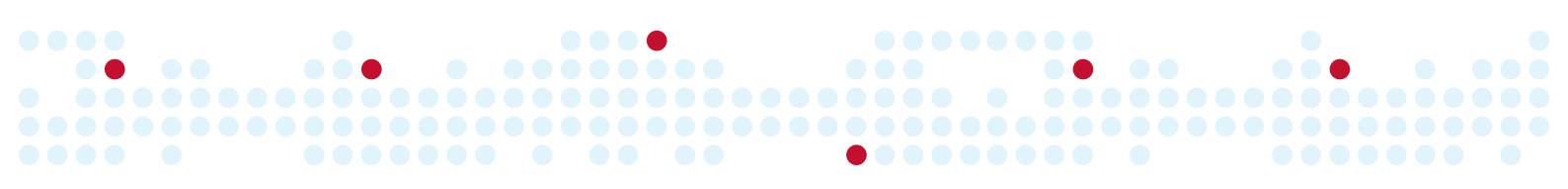
RNase-free filtertips

RNase-free 1.5 ml and 2 ml tubes, (e.g. Non-Stick RNase-free Microfuge Tubes 1.5 ml (AM12450) and 2.0 ml (AM12475) Ambion)

Tabletop microcentrifuge with cooling, capable of 13,000 x g centrifugal force

### Protocol

1. Thaw plasma/serum and MS2 RNA on ice. Samples should be kept on ice until step 6.
2. Cool centrifuge to 4°C.
3. Place 250 µl plasma/serum into a 1.5 ml tube. Spin 3,000g for 5 minutes at 4°C to remove debris.
4. Transfer 200 µl of plasma/serum to a new 1.5 ml tube. (Do not transfer serum coagulate into the purification)
5. Make QIAzol master mix: 800 µl QIAzol + 1.25 µl 0.8 µg/µl MS2 RNA per sample. Vortex briefly to mix.
6. To each 200 µl Plasma/serum sample add 750 µl QIAzol master mix. Vortex.
7. Incubate for 5 minutes at room temperature (RT).
8. Add 200 µl chloroform to each sample. Vortex.
9. Incubate for 2 minutes at RT.
10. Spin at 12,000 g for 15 minutes at 4°C.
11. Heat centrifuge to RT (20-25°C).
12. Transfer upper aqueous phase to 2 ml tube
13. Add 1.5 vol. ethanol. Mix by pipetting
14. Transfer 750 µl to a RNeasy Mini Spin Column, carefully marked.
15. Spin for 30 sec at RT, 13,000 g, discard flow-through
16. Repeat steps 14-15 until all of sample is used – remix by pipetting before loading again.
17. Wash 1x by adding 700 µl RWT, spin for 1 min at RT, 13,000 g, discard flow-through.
18. Wash 3x by adding 500 µl RPE. After each addition spin for 1 min at RT, 13,000 g, discard flow-through.
19. Transfer column to a new, labeled collection tube and spin for 2 min at RT, 13,000 g.
20. Leave the tubes open for 1 min.
21. Transfer column to new Non-Stick RNase-free Microfuge Tube and carefully add 50µl DNase/RNase-free water making sure the liquid is centered on the membrane.
22. Incubate for 1 min. Spin for 1 min at RT, 13,000 g.
23. Store RNA at -80°C.



## RNA Purification from Blood Serum & Plasma - Mouse

### Material

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Extra RPE-buffer, Qiagen, Cat. No. 1018013

MS2 RNA (0.8 µg/µl). Roche, cat. No. 10165948001

Chloroform. Sigma Aldrich, Cat. No. C2432 (4x25 ml)

Abs. Ethanol (99.9%)

RNase-free filtertips

RNase-free 1.5 ml and 2 ml tubes, (e.g. Non-Stick RNase-free Microfuge Tubes 1.5 ml (AM12450) and 2.0 ml (AM12475) Ambion)

Tabletop microcentrifuge with cooling, capable of 15,000 x g centrifugal force

### Protocol

1. Thaw plasma/serum and centrifuge at 3000 x g for 5 min in a 4°C microcentrifuge. Samples should be kept at 4°C or on ice until step 5.
2. Transfer 50 µL of plasma per sample to a new 1.5 mL microcentrifuge tube.
3. Make a QIAzol master mix: 200 µl QIAzol + 0,3 µl 0.8 µg/µl MS2 RNA per sample. Vortex briefly to mix.
4. To each 50 µl Plasma sample add 190 µl QIAzol master mix. Vortex.
5. Incubate for 5 min.
6. Add 50 µL chloroform. Mix by vortexing.
7. Incubate for 2 min.
8. Centrifuge at 12,000 x g for 15 min in a 4°C microcentrifuge.
9. Transfer upper aqueous phase to a new microcentrifuge tube.
10. Add 1.5 volumes of 100% ethanol. Mix thoroughly.
11. Transfer the sample to a Qiagen RNeasy® Mini spin column on a QiaVac Manifold.
12. Pass through the column and wash with 500 µL Qiagen RWT buffer.
13. Wash with 3x 500 µL RPE.
14. Transfer spin-column to a collection tube and centrifuge at 15,000 x g for 2 min at RT.
15. Transfer spin-column to a new microcentrifuge tube, uncap the lid and dry for 1 min.
16. Elute RNA by adding 50 µL RNase-free water to the membrane of the spin column.
17. Incubate for 1 min.
18. Centrifuge at 15,000 x g for 1 min at RT.
19. Store RNA in -80°C freezer.

### microRNA qPCR quantification using the miRCURY LNA™ Universal RT microRNA PCR system

When using Exiqon's qPCR system, cDNA synthesis should be performed in a larger reaction volume than for normal samples. 1µl of eluted mouse RNA in a 40µl RT reaction generally gives a good signal. In order to monitor any contamination, a blank purification (isolation from water) can be included in the RNA isolation.

