Promega

Product Application

miRNA Purification from Plasma Samples

Isolate high quality, amplifiable miRNA from plasma samples using a manual purification system.

Kit: ReliaPrep™ FFPE Total RNA Miniprep System (Cat # Z1002)

Analyses: NanoDrop, 1-Step RT-qPCR, 2-Step RT-qPCR

Sample Type(s): Human Plasma

Input: 300µL of plasma

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be requested by e-mailing Technical Services techserv@promega.com

Materials Required:

- ReliaPrep™ FFPE Total RNA Miniprep System (Cat# Z1002)
- Proteinase K (Cat# MC5005)
- Microcentrifuge
- 100% Isopropanol

Protocol:

1. Add 80µL of Proteinase K to 300µL of plasma in a 1.5mL microcentrifuge tube.
2. Add 230µL of BL Buffer to the sample.
3. Incubate the sample at 56°C for 15 minutes.
4. Allow the tubes to cool down to room temperature for 2 minutes.
5. Prepare DNase treatment mix as follows:
    - 13µL $MnCl_2$ 0.09M
    - 7µL DNase Buffer
    - 10µL DNase I Enzyme
6. Add 30µL of DNase treatment mix to the sample using gentle pipetting or vortex for 5 seconds to mix.
7. Incubate the sample at room temperature for 15 minutes.
8. Add 780µL of 100% isopropanol. Vortex for 15 seconds.
9. Transfer 750µL of solution into a Binding Column/Collection Tube assembly.
10. Centrifuge the assembly at 10,000 x g for 30 seconds. Discard the flow-through.
11. Repeat steps 8-9 until all the solution has been passed through the Binding Column/Collection Tube assembly.
12. Add 500µL of 1X Wash Solution (with ethanol added) to the binding column.
13. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through.
14. Add 500µL of 1X Wash Solution to the Binding Column.
15. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through.
16. Centrifuge the Binding Column/Collection Tube assembly at 16,000 x g for 3 minutes to dry.
17. Transfer the Binding Column to a clean Elution Tube.
18. Add 50µL of Nuclease-Free Water.
19. Centrifuge at 16,000 x g for 1 minute.

Results: miRNA was purified from human plasma collected from 4 individuals. The recovery of total RNA purified from 300µL of plasma using the reported miRNA from plasma protocol was compared to the recovery of total RNA purified from 200µL of plasma using the Qiagen miRNeasy Serum/Plasma Kit.

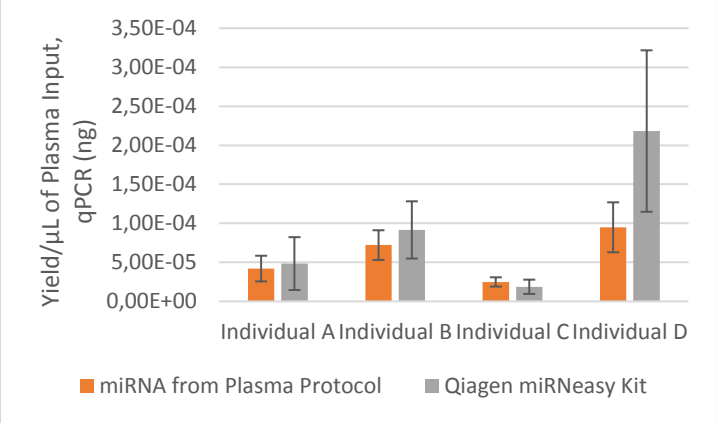

Figure 1. RNA yield per microliter of input resulting from purification of total RNA from plasma using the miRNA from plasma protocol and the Qiagen miRNeasy Serum/Plasma Kit quantified by 1-step RT-qPCR with primers for the B2M transcripts. Following total RNA extraction, samples were analyzed by 1-step RT-qPCR, and relative concentrations were determined based on a standard curve prepared with universal human RNA that was run on the same plate. Mean ± Standard Deviation of n=3 is shown.

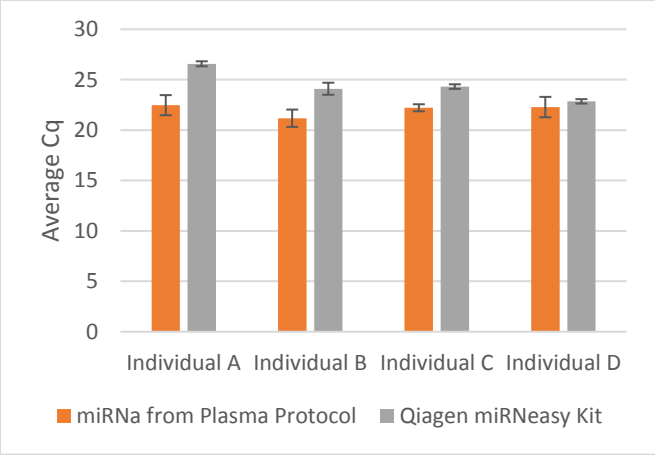

Figure 2. Levels of the miR-16 miRNA were compared by 2-step RT-qPCR for samples extracted using the reported method for purification of total RNA from plasma and the Qiagen miRNeasy Serum/Plasma Kit. Cq values are reported for the 2-step RT-qPCR amplification of miR-16. The Cq values have been mathematically normalized for differences in sample input volume and resulting volume of eluate, assuming 100% reaction efficiency. Mean ± Standard Deviation of n=3 is shown.

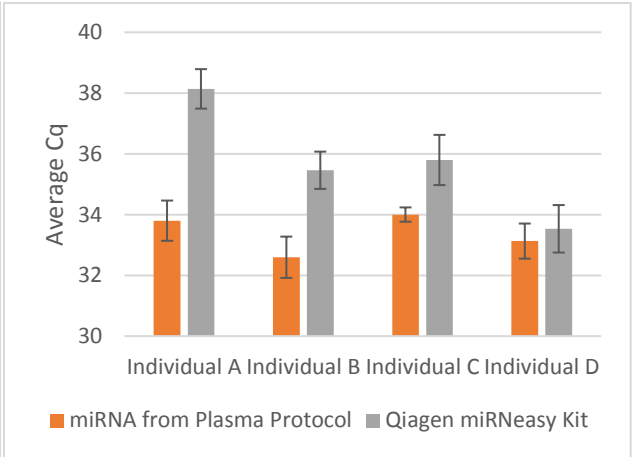

Figure 3. Levels of the let-7a miRNA were compared by 2-step RT-qPCR for samples extracted using the reported method for purification of total RNA from plasma and the Qiagen miRNeasy Serum/Plasma kit. Cq values are reported for the 2-step RT-qPCR amplification of let-7a. The Cq values have been mathematically normalized for differences in sample input volume and resulting volume of eluate assuming 100% reaction efficiency. Mean ± Standard Deviation of n=3 is shown.