Supplementary Material 1

1. Material

Reagents and consumables

Materials	Company	Cat. #
TRIZOL LS reagent	Invitrogen	10296-010
Nuclease-free water	Promega	P1193
Chloroform	Beijing Chemical Works	
Isopropyl alcohol	Beijing Chemical Works	
Ethanol	Beijing Chemical Works	
Escherichia coli polyA polymerase	NEB	M0276S
M-MLV Reverse Transcriptase	Promega	M1705
Recombinant RNasin ribonuclease inhibitor	Promega	N2511
dNTP mix	SIGMA	D7295
1.5 mL EP tubes	Axygen	MCT-150-C
0.2 mL EP tubes	Axygen	PCR-02-L-C
1 mL Rnase free tip	Axygen	T-1000-B-R-S
200 μL Rnase free tip	Axygen	T-200-B-R-S
10 μL Rnase free tip	Axygen	T-300-B-R-S
MicroRNA primer system	MystiCq microRNA qPCR assay primer	
Solutions		
1. PBS 500 mL		
1) KCl	0.1 g	
2) KH_2PO_4	0.1 g	
3) NaCl	4.0 g	
4) $Na_2HPO_4.12H_2O$	1.4425 g	

1. Dissolve the above components in ddH2O and adjust to pH 7.4 with 0.1 N NaOH. Add ddH2O to final volume of 500 mL. Autoclaved and stored at 4℃.
2. 75% ethanol (v/v)
   75% ethanol (v/v) should be prepared freshly by mix ethanol with nuclease-free water.

2. Methods

1) RNA isolation by TRIZOL LS regent

Homogenizing samples

Add 0.75 mL of TRIZOL LS Reagent per 0.25 mL serum sample and 1 μL (20 nM) synthesized internal control 1 miRNA per serum sample.

Homogenize by pipetting or alternative vortexing.

Phase separation

Incubate the homogenized samples for 5 minutes at room temperature.

Add 0.2 mL of chloroform per 0.75 mL of TRIZOL LS reagent. Cap sample tubes securely.

Shake tubes vigorously by hand for 15 seconds and incubate them at room temperature for 5 to 10 minutes. Centrifuge the samples at no more than 12,000 ×g for 15 minutes at 2℃ to 8℃.

Sample will separate in 3 layers- Phase separation.

a. Top layer clear aqueous phase = RNA

b. Middle layer white cloudy phase = DNA

c. Bottom layer red phenol phase = protein

Carefully transfer the aqueous phase to a clean 1.5 mL nuclease-free EP tube.

RNA precipitation

Add as much isopropanol as the amount of aqueous phase.

Homogenize the aqueous solution by vortexing or flicking.

Incubate samples at –20℃ for more than 30minutes and centrifuge at no more than 12,000 ×g for 15-30 minutes at 2℃ to 8℃.

RNA wash and resuspension

Remove the supernatant.

Wash the RNA pellet once with 75% ethanol, adding at least 1ml of 75% ethanol per 0.75 mL of TRIZOL LS reagent used for the initial homogenization.

Mix the sample by vortexing and centrifuge at no more than 7,500 ×g for 5 minutes at 2℃ to 8℃.

Briefly dry the RNA pellet.

Dissolve RNA in 15 μL RNase-free water by passing the solution a few times through a pipette tip.

Determine the quality and quantity of microRNA on Nanadrop 8000.

2) First strand cDNA synthesize

A-Plus

Please use A-Plus bacterial polyA polymerase to add polyA tail for pre- and mature form of miRNAs.

For 20 μL reaction, add:

RNA	10 pg-1 μg
10× Buffer	2 μL

dATP (10 mM)	2 μL
PolyA polymerase	0.5 μL
RNase inhibitor	0.5 μL
RNase-free water	
Total	20 μL

Incubate at 37℃ for 1 hr

RT-PCR

Take the reaction tube out of PCR machine and add 1 μL of 0.5 μg/μL RT primer, incubate at 70℃ for 5 minutes, then place on ice immediately and keep it for at least 2 minutes (The major purpose of this step is to disrupt the secondary structure of RNA and primer).

In a 20 μL reaction, add:

5× Buffer	4 μL
dNTP(10mM)	1 μL
M-MLV	0.5 μL
RNase inhibitor	0.5 μL
A-Plus reaction mix	10 μL
RNase-free water	4 μL
Total	20 μL

Incubate at 42℃ for 1 hr

3. qPCR

In a 25 μL reaction, add:

cDNA	1 μL
10× UPM	2 μL
10× Gene specific primer (10 uM)	2 μL
2× qPCR Mix	10 μL
ROX	0.4 μL
ddH_2O	4.6 μL
Total	20 μL

QPCR system

Instrument: ABI viia@7

Set up:

95℃ 5 min

95℃ 30 sec
60℃ 1 min
} 40 cycles

95℃ 15 sec
60℃ 1 min
} Dissociation stage

95℃ 15 sec