Isolation of extracellular vesicle RNA from serum

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Abstract

Extracellular vesicles can be isolated from different types of body fluids such as serum. These extracellular vesicles may contain protein, mRNA, and non-coding RNA. Extracellular vesicle RNA isolated from serum could be useful as a biomarker of disease. This protocol presents sample collection, process, and extracellular RNA isolation of serum using three different commercially available kits.

Reagents

Reagents:

One of the three commercial kits:

- miCURY RNA Isolation Kit –Biofluids (Exiqon, #300112)
- miRNeasy Mini Kit (Qiagen, #217004)
- SeraMir Exosome RNA Purification Kit (System Biosciences, #RA806TC-1)
- Isopropanol
- Absolute ethanol
- Nuclease free H2O

Equipment

- Serum Tube (BD, 367988)
- 15 ml centrifuge tubes
- Bench top centrifuge
- Vortex Mixer
- Micropipettes with sterile tips 20-1000
- Sterile collection bottle
- Microcentrifuge tubes

Procedure

1. Collect 10ml blood in serum collection tube.
2. Allow blood to clot at room temperature for 1 hour.
3. Centrifuge tube at 3000 rpm for 10s min at room temperature.
4. Aliquot 1 ml serum into microcentrifuge tubes being careful to avoid disturbing the

interface.

5. Store samples at -70°C until ready for use.

6. RNA Isolation can be performed from one of the following options.

A. miCURY RNA Isolation Kit, Exiqon

i. Transfer 500 µl of serum into 1.5 ml microcentrifuge tube.

ii. Add 120 µl Lysis Solution BF, vortex 5 sec, incubate for 3 min at room temperature.

iii. Add 50 µl Protein Precipitation Solution BF, vortex 5 sec, incubate for 1 min at room temperature.

iv. Centrifuge at 11,000g for 3 min at room temperature.

v. Transfer clear supernatant to new microcentrifuge tube.

vi. Add 675 µl Isopropanol, vortex 5 sec.

vii. Assemble microRNA Mini Spin Column BF in a new collection tube and load 700 µl of sample into column.

viii. Centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.

ix. Load remaining sample onto column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.

x. Add 700 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.

xi. Add 250 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 2 min at room temperature. Discard flow-through and return column to collection tube.

xii. Add 50 µl rDNase directly onto membrane of spin column.

xiii. Close lid and incubate for 15 min at room temperature

xiv. Add 100 µl Wash Solution 1 BF to column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.

xv. Add 700 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.

xvi. Add 250 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 2 min at room temperature.

xvii. Transfer spin column into a new collection tube.

xviii. Add 50 µl RNase free H2O directly onto the membrane of spin column.

xix. Close lid and incubate for 1 min at room temperature.

xx. Centrifuge at 11,000g for 1 min at room temperature.

xxi. Discard spin column and store purified RNA at -70°C.

B. miRNeasy Mini Kit

i. Transfer 500 µl of serum into 1.5 ml microcentrifuge tube.

ii. Add 700 µl QIAzol Lysis Reagent, vortex 5 sec, incubate for 5 min at room temperature.

iii. Add 140 µl chloroform, shake vigorously for 15 sec, incubate for 3 min at room temperature.

iv. Centrifuge sample at 12,000g for 15 min at 4°C.

v. Transfer the upper aqueous phase to a new microcentrifuge tube.

vi. Add 500 µl 100% Ethanol, mix by pipetting.

vii. Assemble RNAeasy Mini Column in a new collection tube and load entire sample onto column.

viii. Centrifuge sample at 8,000g for 15 sec at room temperature. Discard flow-through and return column to collection tube.

ix. Add 700 µl Buffer RPE to column and centrifuge at 8,000g for 15 sec at room temperature. Discard flow-through and return column to collection tube.

x. Add 500 µl Buffer RPE to column and centrifuge at 8,000g for 2 min at room temperature. Discard flow-through and return column to collection tube.

xi. Centrifuge column at 8,000 x g for 1 min at room temperature to dry membrane.

xii. Transfer spin column into a new collection tube.

xiii. Add 30 µl RNase free H2O directly onto the membrane of spin column.

xiv. Close lid and centrifuge for 1 min at 8,000 x g at room temperature.

xv. Discard spin column and store purified RNA at -70°C.

C. SeraMir Exosome RNA Purification Kit

i. Transfer 500 µl of serum into 1.5 ml microcentrifuge tube.

ii. Add 100 µl ExoQuick-TC, mix well, incubate for 30 min at 4°C.

iii. Centrifuge sample at 13,000 rpm for 2 min at 4°C.

iv. Remove supernatant, add 350 µl Lysis Buffer to EV, vortex 15 sec, incubate for 5 min at room temperature.

v. Add 200 µl 100% Ethanol, vortex 10 sec.

vi. Assemble Spin Column in a new collection tube and load 700 µl of sample onto column.

vii. Centrifuge sample at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.

viii. Load remaining sample onto column and centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.

ix. Add 400 µl Wash Buffer to column and centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.

x. Add 400 µl Wash Buffer to column and centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.

xi. Centrifuge column at 13,000 rpm for 2 min at room temperature to dry membrane.

xii. Transfer spin column into a new collection tube.

xiii. Add 30 µl Elution Buffer directly onto the membrane of spin column.

xiv. Close lid and centrifuge at 2,000 rpm for 2 min at room temperature.

xv. Increase speed and centrifuge at 13,000 rpm for 1 min at room temperature.

xvi. Discard spin column and store purified RNA at -70°C.