



ExoQuick[®] Exosome Isolation and RNA Purification Kits

Cat # EQ806A-1, EQ806TC-1, EQ808A-1

User Manual

Storage: Please see individual components

Version 2
8/14/2018

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Product Description

RNAs present in patient body fluids and cell culture media are a rich and untapped source of disease-related biomarkers. The RNAs are stable in serum because they are encapsulated in circulating exosomes. Exosomes are 40–100 nm membrane vesicles secreted by most cell types *in vivo* and *in vitro*. Exosomes are found in blood, urine, amniotic fluid, malignant ascites fluid, and cell media, and contain distinct subsets of microRNAs depending upon the tumor or tissue from which they are secreted. The ExoQuick Exosome Isolation and RNA Purification Kits include everything needed to accurately and sensitively measure RNAs from serum samples. Exosomes are efficiently isolated using SBI's ExoQuick/ExoQuick-TC solutions, and the exoRNAs are purified using a phenol-free lysis buffer and rapid spin columns.

List of Components

Components	ExoQuick Exosome Isolation and RNA Purification Kit (for Serum & Plasma) EQ806A-1	ExoQuick Exosome Isolation and RNA Purification Kit (for Tissue Culture Media) EQ806TC-1	ExoQuick Exosome RNA Column Purification Kit EQ808A-1	Storage
ExoQuick or ExoQuick-TC	5 ml	10 ml	N/A	RT
Lysis Buffer	8 ml	8 ml	8 ml	RT
Wash Buffer	20 ml	20 ml	20 ml	RT
Elution Buffer	750 μ l	750 μ l	750 μ l	RT
ExoQuick RNA Columns	20 columns	10 columns	20 columns	RT

Storage

The Kit is shipped on blue Ice and the components should be stored at recommended temperatures as stated above. Properly stored kits are stable for 12 months from the date received.

General Information

- ! **OPTIONAL: Thrombin (Cat. # TMEXO-1) may be added to plasma samples to generate a serum-like solution.**
- ! **If using the ExoQuick Exosome RNA Column Purification Kit (Cat EQ808A-1), please skip to Section B of the protocol.**

Protocol

A. ExoQuick Isolation

1. Collect the biofluid and centrifuge at 3,000 \times g for 15 minutes to remove cellular debris.
2. Transfer the supernatant to a new tube.

! **OPTIONAL: If additional debris remains, centrifuge the supernatant for additional 10 minutes at 12,000 x g and transfer the supernatant to a new tube.**

3. Add the appropriate volume of ExoQuick or ExoQuick-TC to the clarified biofluid as shown in the table.

Biofluid	Sample Volume	ExoQuick Volume	Incubation Time
Serum or Plasma	500 μ l	120 μ l	30 min at 4°C
Tissue Culture Media	5 ml	1 ml	12h-overnight at 4°C

4. Mix well by inverting or flicking the tube and incubate on ice for 30 minutes for serum or 12 hours to overnight for tissue culture media. The tubes do not need to be rotated during the incubation period.

5. Centrifuge the ExoQuick/biofluid mixture at 1,500 x g for 30 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the EVs may appear as a beige or white pellet at the bottom of the tube.

6. Carefully aspirate off the supernatant. Spin down any residual ExoQuick solution by centrifugation at 1,500 x g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated EVs in the pellet.

7. Resuspend exosome pellet in 350 μ l of Lysis Buffer and vortex for 15 seconds.

8. Place at room temperature for 5 minutes to allow complete lysis.

! **OPTIONAL: Add 5 μ l of SeraMir Control spike-in Small RNA (cat# RA805A-1)**

B. Purification of ExoRNA

1. Add 200 μ l of 100% Ethanol to resuspended EVs and vortex for 10 seconds.

2. Take out the ExoQuick RNA column and assemble spin column and collection tube by placing spin column into the collection tube.

3. Transfer sample to spin column and centrifuge at 13,000 rpm for 1 minute.

4. Discard the flow-through and place the column back into the collection tube.

5. To wash the column apply 400 μ l of Wash Buffer and centrifuge at 13,000 rpm for 1 minute. Discard the flow through.

6. Repeat steps 4 – 5 one more time (total of 2 washes).

7. Discard the flow through and centrifuge at 13,000 rpm for 2 minutes to dry.

C. ExoRNA Elution

1. Discard collection tube and assemble spin column with new, RNase-free, 1.5 ml elution tube (not provided).

2. Add 30 μ l Elution Buffer onto the membrane of the spin column and centrifuge at 2,000 rpm for 2 minutes to load the membrane with the buffer.

3. Increase speed to 13,000 rpm and centrifuge for 1 minute to elute the exoRNAs. You should recover 30 – 40 μ l exosomal RNA.

The amount of RNA isolated from exosomes will vary depending upon the starting biofluid or cell type. For a serum sample, the level of RNA isolated from 500 μ l is usually in the 1-10ng range and can be measured using Agilent Bioanalyzer chip. For a tissue culture media sample, the level of RNA isolated will vary depending on the cell type and growth confluency.

Example Data and Applications

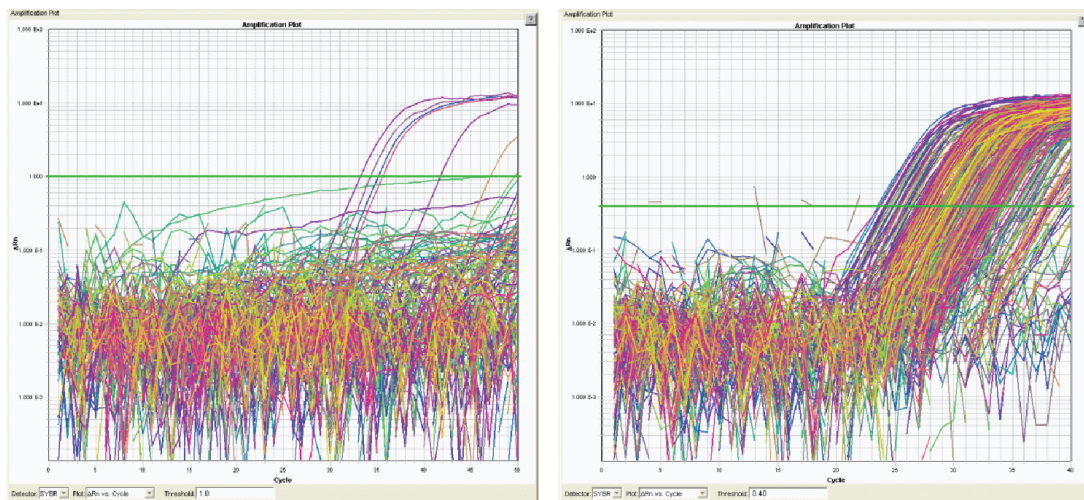


Figure 1. Serum RNA prepared by the ExoQuick Exosome Isolation and RNA Purification Kit delivers more reliable, reproducible qPCR profiles than when the RNA is isolated using conventional Trizol methods. Profiling of 380 human microRNAs using SBI's Complete SeraMir Exosome RNA Amplification and Profiling Kit (Cat #RA820A-1). The phenol-free exosome lysis step coupled to the small RNA binding columns isolates exoRNAs with much higher purity than Trizol/Phenol based methods. The exoRNAs are compatible with downstream polyadenylation and reverse transcription reactions for amplification and accurate qPCR profiling.

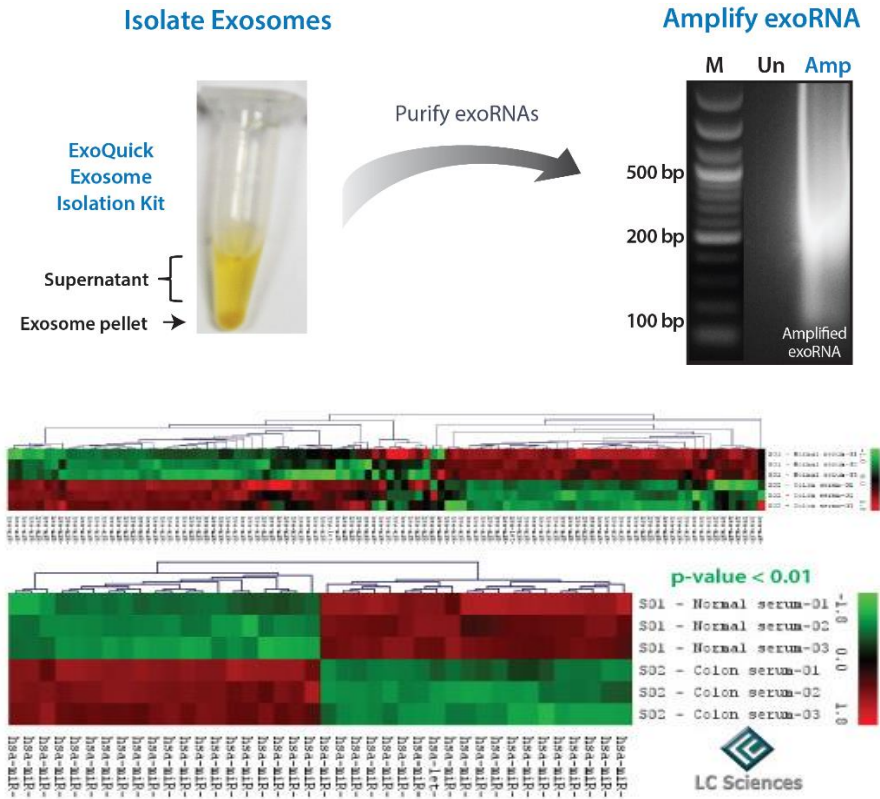


Figure 2. Serum exoRNAs prepared using ExoQuick Exosome Isolation and RNA Purification Kit deliver excellent performance in microarray studies. Samples from a pooled normal serum preparation and from a male caucasian (age 73) with adenocarcinoma of the colon were used in this study. Exosomes were precipitated from 250 μ L of serum using the ExoQuick Exosome Isolation and RNA Purification Kit. The T7-amplified “sense” exoRNAs were then used for direct labeling analyses on LC Sciences miRBase ver.16 array chips (performed in triplicate). The exoRNAs were hybridized across 1,214 different microRNAs on the probe set. Of the 1,214 microRNAs analyzed, 79 microRNAs showed a signal intensity >32 . Within this set of 79, there was a clear colon versus normal “signature set” of 40 microRNAs that could discriminate normal from colon cancer serum samples with a p-value < 0.01 . The identities of the microRNAs found in this study have been masked while further investigation continues.

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our website: <http://www.systembio.com>

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