



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

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the License and Warranty Statement contained in this user manual.

Contents

Product Description.....	2
List of Components.....	2
Storage.....	2
General Information.....	2
Protocol.....	2
Example Data and Applications.....	4
Technical Support.....	6
Licensing and Warranty Statement.....	6

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 µl	750 µl	750 µl	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 × 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

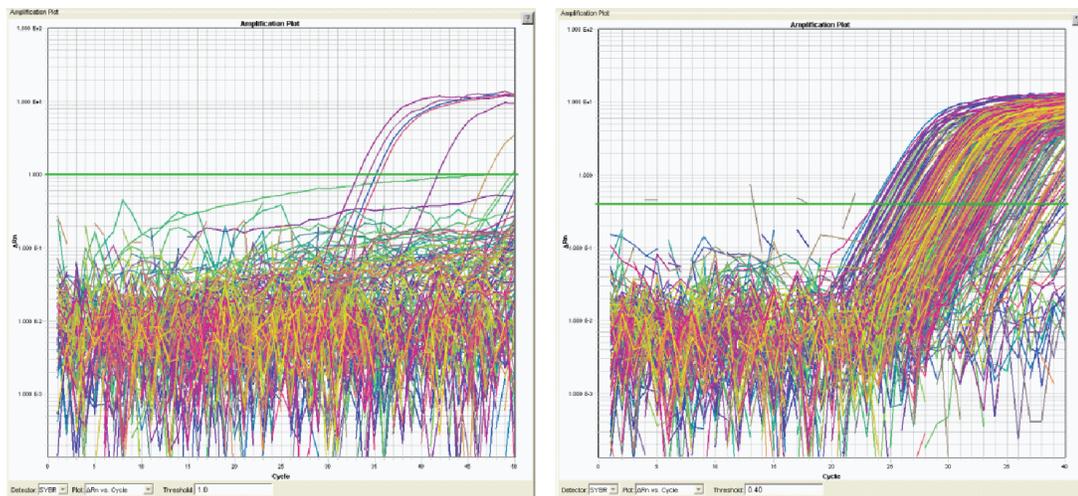


Figure 1. Serum RNA prepared by the ExoQuick Exosome Isolation and RNA Purification Kit delivers more reliable, reproducible qPCR profiles than when the RNA is isolated using conventional Trizol methods. Profiling of 380 human microRNAs using SBI's Complete SeraMir Exosome RNA Amplification and Profiling 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.

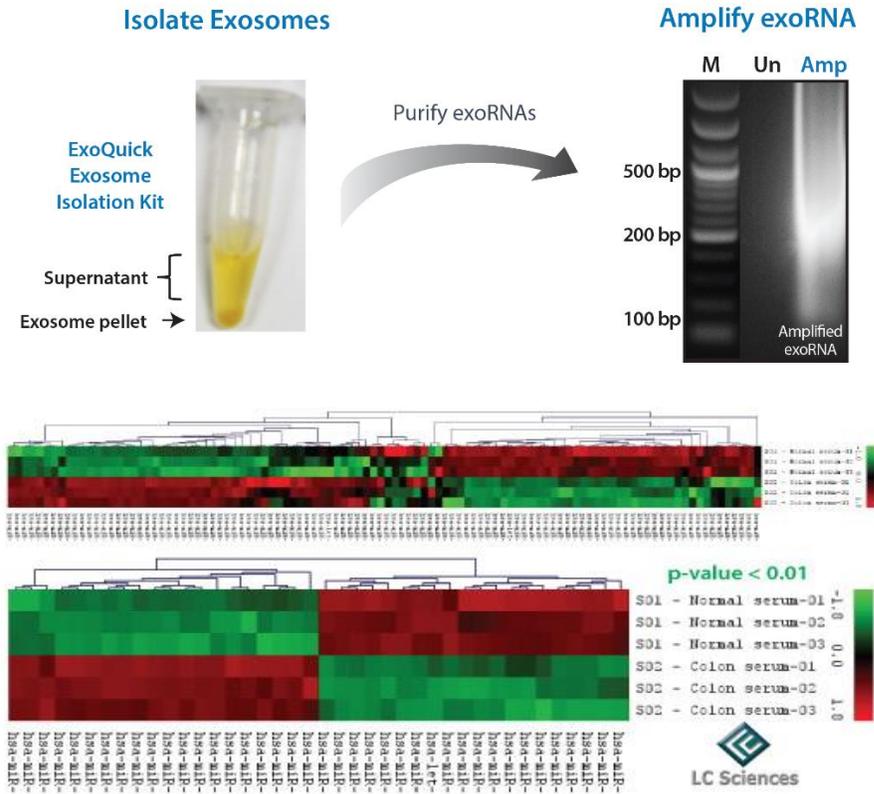


Figure 2. Serum exoRNAs prepared using ExoQuick Exosome Isolation and RNA Purification Kit deliver excellent performance in microarray studies. Samples from a pooled normal serum preparation and from a male caucasian (age 73) with adenocarcinoma of the colon were used in this study. Exosomes were precipitated 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>

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 9682200

Toll-Free: (888) 266-5066

Fax (650) 968-2277

E-mail:

General Information: info@systembio.com

Technical Support: tech@systembio.com

Ordering Information: orders@systembio.com

Licensing and Warranty Statement

Limited Use License

Use of the ExoQuick Exosome Isolation and RNA Purification Kits (*i.e.*, the “Products”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Products to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Products constitutes acceptance of the above terms.

The purchaser of the Products is granted a limited license to use the Products under the following terms and conditions:

- The Products shall be used by the purchaser for internal research purposes only. The Products are expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Products may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Products should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the Products does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Products for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Products in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Products meet the specifications described in this manual. If it is proven to the satisfaction of SBI that the Products fail to meet these specifications, SBI will replace the Products or provide the purchaser with a refund. This

limited warranty shall not extend to anyone other than the original purchaser of the Products. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Products.

SBI's liability is expressly limited to replacement of Products or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Products, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Products for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

© 2018 System Biosciences (SBI), All Rights Reserved

This page is intentionally left blank

This page is intentionally left blank



System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

E-mail:

General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com