EVeryRNA™ EV RNA Purification System with ExoQuick EV Isolation

Cat # EVery106EQ-1

User Manual

Storage:
DNase I and Glycogen at -20°C.
All other components at room temperature

Version 1
8/18/2020

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Product Description

Discover more when you capture total EV RNA

Combine the time- and labor-savings of ExoQuick EV Isolation technology with the total RNA isolation capabilities of EVeryRNA EV RNA Purification technology for a complete solution for your EV RNA biomarker workflows.

The EVeryRNA EV RNA Purification System overcomes many of the challenges faced when isolating RNA from extracellular vesicles (EVs), most notable is the ability to capture total EV RNA, including small RNAs. EVeryRNA is effective even with low amounts of input RNA and is capable of delivering high yields of highly pure RNA. Because the RNA elutes in a small sample volume, thus generating a highly concentrated prep, you can increase the amount of RNA used in a single downstream reaction for better data coverage quality.

- Move quickly and confidently with exoRNA isolation that's high-yield and complete in <30 minutes
- Find what others miss when you capture every RNA with EVeryRNA
- Achieve phenol-level yields with a safer column-based method
- Get more RNA for each downstream reaction with EVeryRNA's small-volume elutions
- Isolate RNA from EVs for a full range of downstream applications, such as RNA-seq and miRNA profiling
- Ensure delivery of highly pure RNA by using the included DNaseI
- Maximize productivity with ExoQuick EV Isolation bundled with EVeryRNA

The EVeryRNA EV RNA Purification System with ExoQuick EV Isolation comes with sufficient reagents to perform 20 EV isolation reactions from serum, plasma, or ascites fluid and then EV RNA purification. EVeryRNA technology is available bundled with several of SBI's powerful EV isolation technologies as well as a cDNA Synthesis and Pre-amplification Kit (Table 1).

Table 1. EVeryRNA EV RNA Purification Products

Catalog number	Description
EVery100A-1	EVeryRNA™ EV RNA Purification System
EVery106EQ-1	EVeryRNA™ EV RNA Purification System with ExoQuick ™EV Isolation
EVery106TC-1	EVeryRNA™ EV RNA Purification System with ExoQuick-TC EV Isolation
EVery106SS-1	EVeryRNA™ EV RNA Purification System with SmartSEC™ Single
EVery200A-1	EVeryRNA™ cDNA Synthesis & Pre-Amplification Kit
EVery300A-1	EVeryRNA™ EV RNA Purification System & cDNA Synthesis Kit (includes EVery100A-1 and EVery200A-1)

The Workflow: Maximize productivity with ExoQuick and EVeryRNA

Step 1: Isolate EVs with ExoQuick

Isolating EVs from plasma, serum, and ascites fluid with ExoQuick is quick and easy, with a simple workflow that involves minimal hands-on time and low input sample volume requirements (Figure 1). ExoQuick is an excellent option for researchers who need to purify multiple exosome samples and/or samples from small animal models or clinical research samples.

To isolate exosomes from cleared serum, plasma, or ascites fluid, simply:

- Add an appropriate volume of ExoQuick to as little as 100 µL sample
- Incubate for at least one hour at 4°C
- Isolate exosomes with a 30-minute low-speed spin (1500 *g*)

Isolated exosomes can be found in the pellet and resuspended in an appropriate solution.

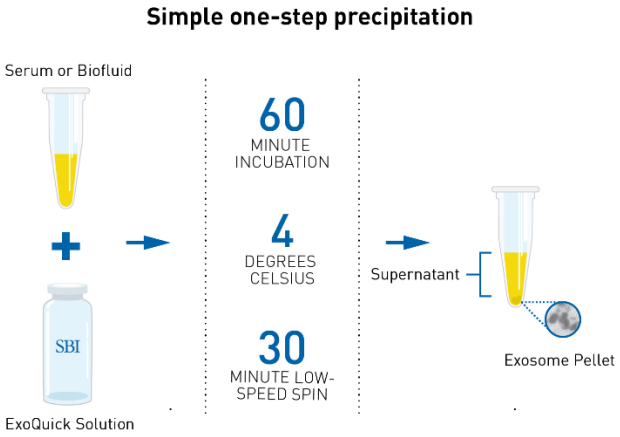


Figure 1. The ExoQuick workflow.

Step 2: Purify EV RNA with EVeryRNA

The EVeryRNA EV RNA Purification System delivers high yields of highly concentrated RNA from already isolated EVs. The column-based workflow is easy to implement and can be completed in less than 30 minutes (Figure 2).

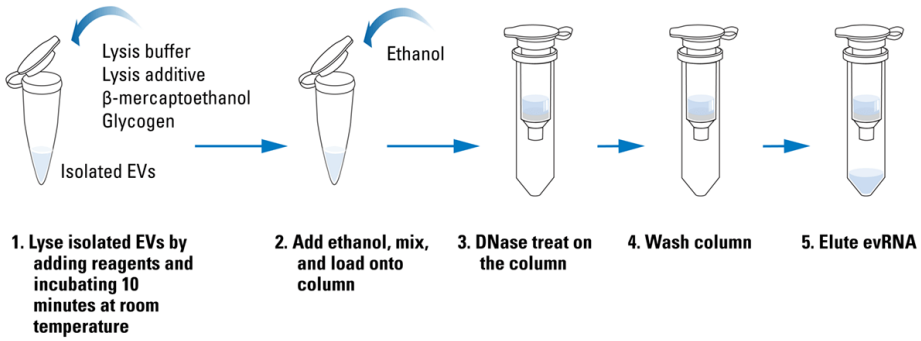


Figure 2. The quick and easy EVeryRNA EV RNA purification workflow.

List of Components

Table 2. Components of EVery100A-1, EVeryRNA™ EV RNA Purification System

Components	Qty/Volume	Storage Temperature
ExoQuck	5 ml	RT
Lysis buffer	25 ml	RT
Lysis additive	3.5 ml	RT
Glycogen	100 µL	-20˚C
Wash solution	18 ml	RT
Elution solution	1 ml	RT
Micro spin column	20	RT
Collection tubes	20	RT
Elution Tubes	20	RT
RNase free DNase I	200 µL	-20˚C
Enzyme buffer	1.5 ml	RT

NOTE: The table above is for 20 reaction kit.

Additional Required and Optional Equipment Not Included in Kit

1. 96-100% Ethanol
2. β-mercaptoethanol (cat# M3148-25ML, Sigma)- optional, but highly recommended

Protocol

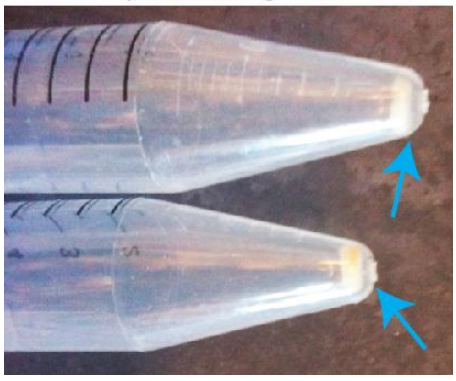
Step 1: EV isolation with ExoQuick™

1. Collect biofluid (e.g. serum or ascites fluid) and centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick Exosome Precipitation Solution to the biofluid. Some examples are shown in the Table below. Mix well by inverting or flicking the tube.

Incubation Time	Biofluid	Sample volume	ExoQuick volume
30 minutes	Serum	250 µL	63 µL
Overnight	Ascites fluid	250 µL	63 µL

3. Refrigerate overnight (at least 12 hours) for ascites fluid or 30 minutes for serum at +4°C. The tubes should not be rotated or mixed during the incubation period and should remain upright.
4. Centrifuge ExoQuick/biofluid mixture at 1500 × g for 30 minutes. Centrifugation may be performed at either room temperature or +4°C with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the vessel.

Exosome pellets obtained from 10 ml of cerebral spinal fluid using ExoQuick.



5. Aspirate supernatant. Spin down residual ExoQuick solution by centrifugation at 1500 × *g* for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated exosomes in pellet.
6. Resuspend exosome pellet in 100-500 µL using sterile 1X PBS, or specific buffer according to your downstream application. We recommend using the precipitated exosomes immediately rather than freezing them for future use.

Step 2: Purification of EV RNA with EVeryRNA

Before you start the protocol for exosomal RNA isolation:

1. The protocol is outlined for 500 µL of sample input. The pellet from **ExoQuick** should be resuspended in 1xPBS to 500 µL to prevent column clogging during isolation.
2. All steps should be performed at room temperature and all centrifugation steps performed at 3,300 x g.
3. It is highly recommended to warm up **Lysis Buffer** at 60°C for 5-10 minutes and mix well until the solution becomes clear again if precipitates are present.

   ! OPTIONAL (highly recommended)

   The use of β-mercaptoethanol in the Lysis Buffer is highly recommended. Add 10 µL of β-mercaptoethanol to each 1 mL of **Lysis buffer**.

   ! OPTIONAL (highly recommended)

   Add 5 µL of Glycogen to the Lysis Mix if you are expecting a low RNA yield.

4. Prepare a working solution of the **Wash solution** by adding 42 mL of 96-100% ethanol to the supplied bottle containing the concentrated Wash Solution.
5. The RNA yield may be increased by using **Elution Buffer** warmed to 60°C.

RNA isolation steps:

1. Add 700 µL of **Lysis Buffer**, 110 µL of **Lysis Additive** and 7 µL of β-mercaptoethanol (optional) to the 500 µL PBSx1 Buffer containing the purified exosomes.
2. Mix well by vortexing for 10 sec. then incubate at RT for 10 min.

   ! OPTIONAL (highly recommended)

   Add 5 µL of Glycogen to the lysis mix if you know that RNA yield is low.

3. After incubation add 1.35 mL of 96%-100% EtOH to the mix from Step3 and mix well by vortexing for 10 seconds.
4. Transfer 750 µL of the mixture from Step 4 into a Micro Spin column. Centrifuge for 1 minute. Discard the flowthrough and reassemble the spin column in its collection tube.
5. Repeat Step 5 four times to transfer the remaining mixture from Step 4 into the Micro Spin column.
6. Apply 400 µL of **Wash Solution** on the column and centrifuge for 2 minutes. Discard the flowthrough and reassemble the spin column in its collection tube.

   ! OPTIONAL (highly recommended)

   On-column DNA removal:

   a. For every on-column reaction prepare a mix of 7.5 µL of **RNase-free DNase I** and 50 µL of **Enzyme buffer**. Mix gently by inverting the tube a few times or flicking the tube with your fingers to mix.

! **DO NOT VORTEX**

b. Apply 57 µL of DNase I mix from step a. to the column and incubate at 25°C-30°C for 15 minutes.

7. Apply 600 µL of **Wash solution** to the column and centrifuge for 30 seconds at 3,300g. Discard the flowthrough and reassemble the spin column in its collection tube.
8. Repeat step 9 one more time, for a total of two washes.
9. Centrifuge the empty column for 1 minute to completely remove any residual solution. Discard the collection tube.
10. Transfer the spin column to a fresh Eppendorf tube. Apply 10 µL to 25 µL of **Elution solution** to the column and let it stand for 2 minutes. Centrifuge for 1 minute at 3,300g to elute.
11. To maximize the recovery of the RNA, add the eluate collected from step 12 back on the column, let it stand for 2 minutes. Centrifuge for 1 minute at 3,300g to elute.
12. Exosomal RNA is now ready for downstream applications.

Example Data and Applications

Characterizing ExoQuick EVs with NanoSight

EVs purified with ExoQuick from serum show the expected particle size distribution and high concentration yields when analyzed using NanoSight's Nanoparticle Tracking Analysis (NTA, Figure 3).

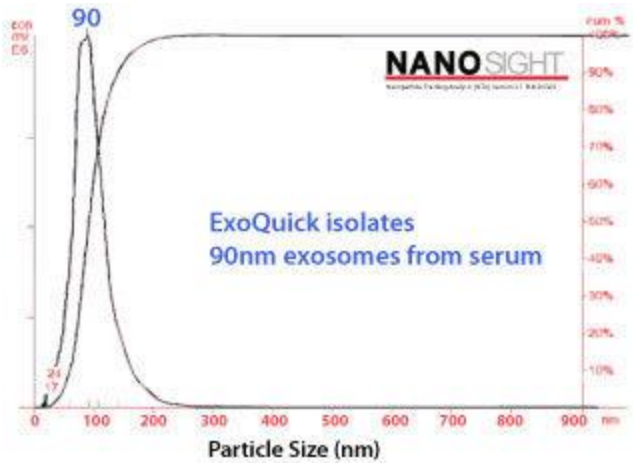


Figure 3. Exosome size distribution and yields from serum. *Exosomes were purified from 50 pooled samples of normal human serum. 250 µL of serum was combined with 63 µL of ExoQuick, incubated at 4°C for thirty minutes, and pelleted by a 1500g spin for thirty minutes. The exosome pellet was resuspended in 100 µL of PBS, diluted 1:10,000, and visualized on the NanoSight LM10 instrument. The analysis shows that the ExoQuick isolation method recovered 90 nm exosomes at a concentration of of 2.74 x 1012 particles/ml.*

EVeryRNA captures Everything

To demonstrate the ability of the EVeryRNA EV RNA Purification System to capture the full range of RNAs, we used the kit to isolate RNA from 10,000 cells (Figure 2, lane 1), from EVs that were isolated from 250 µL of serum using **SmartSEC Single** (Figure 4, lane 2), and from buffer spiked with 0.1 pmol of Cel-miR-39 (Figure 4, lane 3). The high quality of the isolated RNA can be seen in lane 1, where the RNA integrity number (RIN) is 9.9 and the 28S/18S RNA ratio is 1.5. The multiple bands in lane 2 demonstrate that EVeryRNA captures RNAs of different lengths—EVerything—from EVs with no apparent bias or size preference. The strong signal from the spiked-in miRNA in lane 3 demonstrates the good recovery of even small RNAs.

Figure 4. EVeryRNA captures EVerything.

EVeryRNA works well with ExoQuick, yielding broad and unbiased size distribution of RNAs (Figure 5).

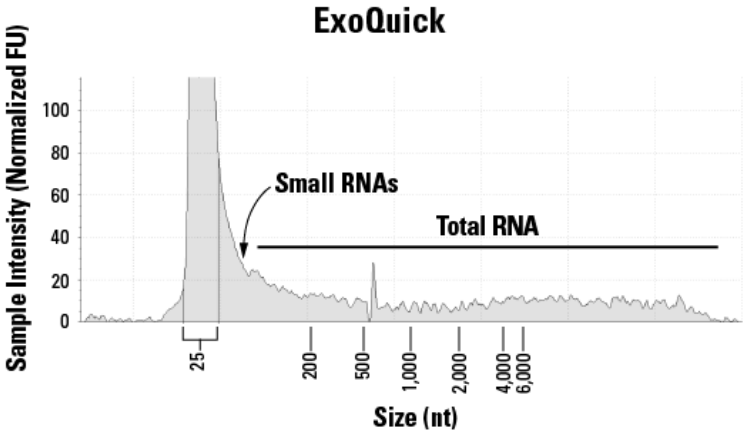


Figure 5. EVeryRNA is compatible with EVs isolated using ExoQuick.

EVeryRNA delivers similar amounts of RNA as phenol-based methods

To demonstrate the excellent RNA yields and robust cDNA synthesis obtained with EVeryRNA, we isolated EVs from 250 µL of serum using **SmartSEC Single**, spiked in 0.1 pmol of Cel-miR-39, and used both the **EVeryRNA EV Purification System** and a phenol-based kit to isolate RNA. The isolated RNA was reverse transcribed using the EVeryRNA cDNA Synthesis & Pre-amplification Kit and the copy number of Cel-miR-39 measured (Figure 6). The EVeryRNA EV Purification System delivered similar levels of Cel-miR-39 as the phenol-based method.

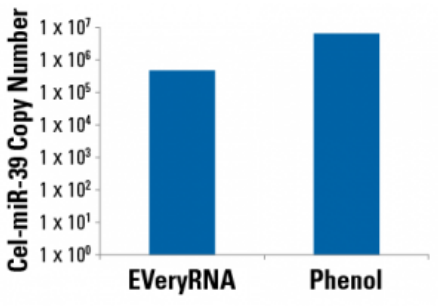


Figure 6. EVeryRNA is EVery bit as good as phenol.

EVeryRNA efficiently isolates mRNA

We used the EVeryRNA EV RNA Purification System and EVeryRNA cDNA Synthesis & Pre-amplification Kit to isolate mRNA and synthesize cDNA from cells overexpressing eGFP (Figure 7). Robust levels of eGFP mRNA are recovered and converted to cDNA when cells are overexpressing eGFP.

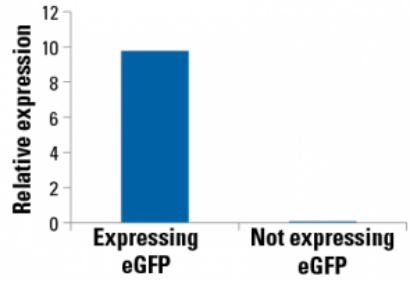


Figure 7. EVeryRNA efficiently isolates longer RNAs like mRNA

miRNA isolated and converted to cDNA using EVeryRNA can be used for miRNA profiling

We isolated EVs from 250 µL of serum using SmartSEC Single and used the EVeryRNA EV RNA Purification System and EVeryRNA cDNA Synthesis & Pre-amplification System to isolate and reverse transcribe EV RNAs for miRNA profiling using the SeraMir Human Exosome RNA Profiling Plate. We were able to detect a number of miRNAs both with amplification (96 miRNAs) and without amplification (16 miRNAs, with 14 overlapping with the miRNAs detected with amplification, Figure 8).

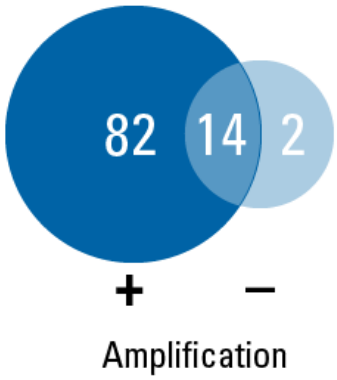


Figure 8. EVeryRNA generates high-quality RNA suitable for miRNA profiling both with and without amplification.

We were also able to show robust, successful RNA-seq runs using RNA isolated from EVs with EVeryRNA (Table 3). All three EV isolation methods tested generated high-quality RNA-seq data.

Table 3. Successful RNA-seq with EVeryRNA-isolated EV RNA

EV Isolation Method	Amount of RNA isolated (ng)	Number of reads	FAST-QC
ExoQuick	4.3	53,349,528	Passed
ExoQuick Ultra	5.4	107,154,128	Passed
SmartSEC Single	5.2	98,886,924	Passed

Technical Support

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

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- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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