EVeryRNA™ EV RNA Purification System

Cat # EVery100A-1

User Manual

Storage:
Store RNase-free DNase I and Glycogen at -20°C
Store all other reagents at room temperature

Version 1
8/18/2020

Contents

Product Description **2**

List of Components **3**

Additional Required and Optional Equipment Not Included in Kit **3**

Protocol **4**

Example Data and Applications **5**

Technical Support **8**

Licensing and Warranty Statement **8**

Product Description

Discover more when you capture total EV RNA

Overcoming many of the challenges with RNA isolation from extracellular vesicles (EVs), the EVeryRNA EV RNA Purification Kit is able 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
- Ensure delivery of highly pure RNA by using the included DNaseI
- Compatible with most downstream applications, including RNA-seq and miRNA profiling
- Works with EVs isolated with commonly-used methods, such as ExoQuick, SmartSEC, and ultracentrifugation.

The EVeryRNA EV RNA Purification System can be used with EVs isolated by commonly used methods, including ExoQuick, SmartSEC, and ultracentrifugation, and comes with sufficient reagents to perform 20 purification reactions. EVeryRNA technology is also available bundled with 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 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 1).

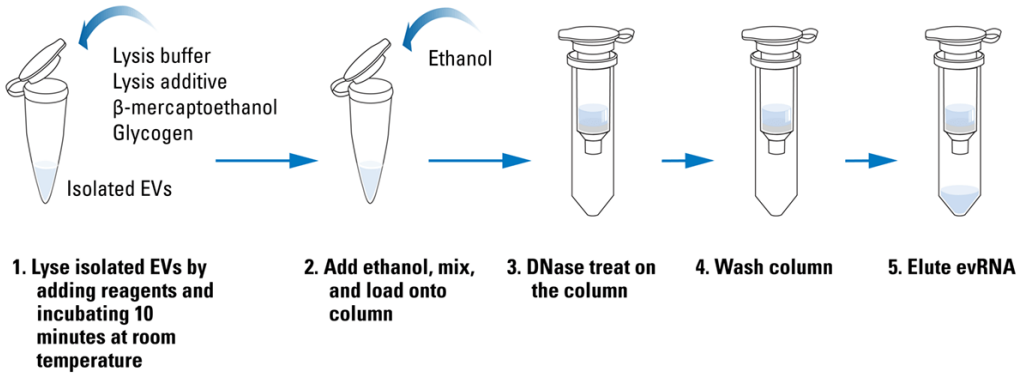

Figure 1. The quick and easy EVeryRNA EV RNA purification workflow.

List of Components

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

Components	Qty/Volume	Storage Temperature
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 the 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

Before you start the protocol for exosomal RNA isolation:

1. The protocol is outlined for 700 µL (**SmartSEC Single**) or 500 µL (**ExoQuick, ExoQuick TC, ExoQuick ULTRA, ExoQuick TC ULTRA**) of sample input. If processing a sample volume lower than 700 µL (500 µL), simply bring the volume of your sample up to 700 µL (500 µL) using 1X PBS and proceed as outlined below. The pellet from **ExoQuick** or **ExoQuick TC** 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 1 mL (700 µL) of **Lysis Buffer**, 150 µL (110 µL) of **Lysis Additive** and 10 µL (7 µL) of β-mercaptoethanol (optional) to the 700 µL (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 1.85 mL (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

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 2, lane 2), and from buffer spiked with 0.1 pmol of Cel-miR-39 (Figure 2, 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.

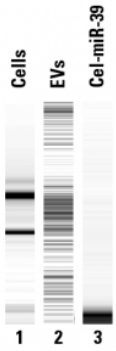

Figure 2. EVeryRNA captures EVerything.

The compatibility of multiple EV isolation techniques with EVeryRNA and the excellent size distribution of RNAs isolated from those EVs is shown in Figure 3.

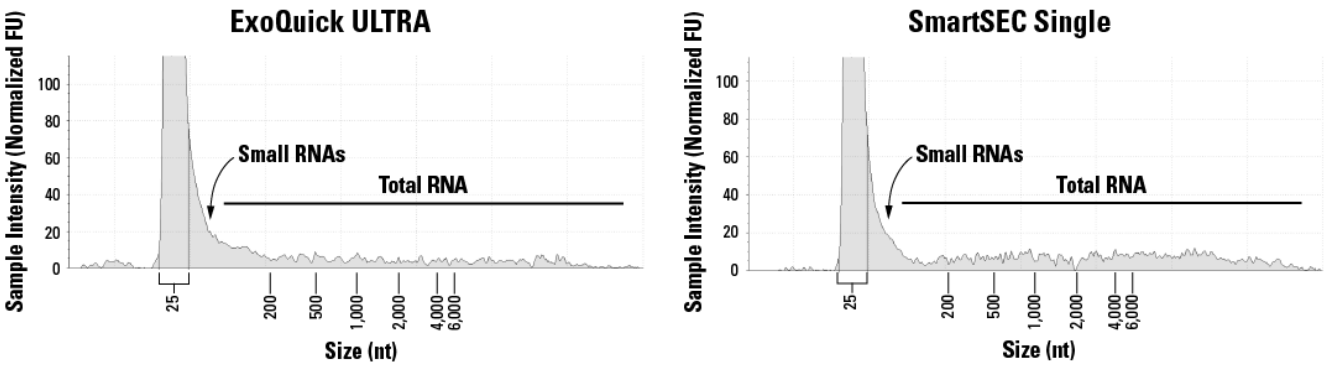

Figure 3. EVeryRNA is compatible with EVs isolated using ExoQuick Ultra and SmartSEC Single.

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 4). The EVeryRNA EV Purification System delivered similar levels of Cel-miR-39 as the phenol-based method.

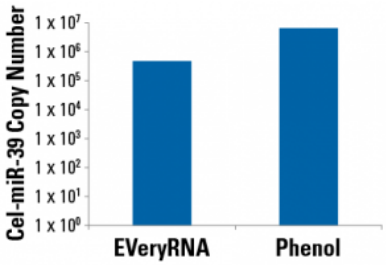

Figure 4. EVeryRNA is EVery bit as good as phenol.

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 (Figure5). Robust levels of eGFP mRNA are recovered and converted to cDNA when cells are overexpressing eGFP.

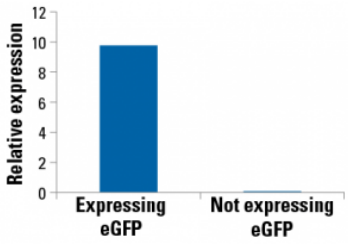

Figure 5. EVeryRNA efficiently isolates longer RNAs like mRNA

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 6).

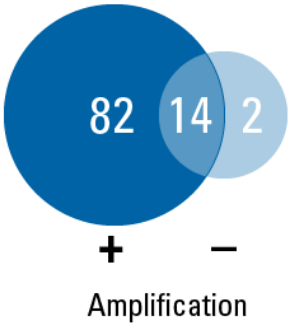

Figure 6. EVeryRNA generates high-quality RNA suitable for miRNA profiling both with and without amplification.

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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Licensing and Warranty Statement

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

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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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