EVery EV RNA Isolation Kit

Cat # EVery100B-1

User Manual

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

Version 1
8/25/2022

Contents

Product Description

Discover more when you capture total EV RNA

EV samples vary drastically in their purity and concentration. Some EV samples from sera of diseased subjects and plasma bring up the challenge of heavy load of contaminating proteins that can potentially lead to column clogging thus lower yields. New column dimensions work just as smoothly for RNA isolation from all types of EV samples, irrespective of upstream EV isolation methods. Overcoming many of the other challenges with RNA isolation from extracellular vesicles (EVs), EVery EV RNA Isolation Kit is able to capture total EV RNA, including small RNAs. It is effective even with low amounts of input RNA and is capable of delivering high yields of highly pure RNA. Move quickly and confidently with exoRNA isolation that's high-yield and completed in <30 minutes.

- Find what others miss when you capture every RNA
- Achieve phenol-level yields with a safer column-based method
- Get more RNA for each downstream reaction
- Ensure delivery of highly pure RNA by using the included DNase I
- Compatible with most downstream applications, including RNA-seq and miRNA profiling

EVery EV RNA Isolation Kit 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.

EVery EV RNA Isolation Kit 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).

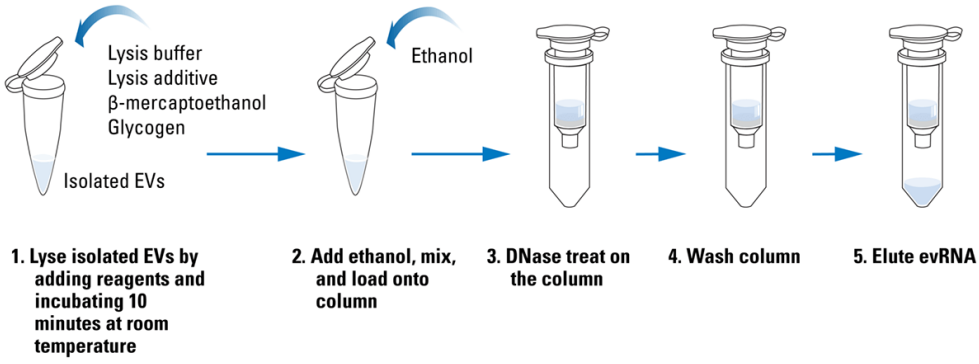

Figure 1. The quick and easy EVery EV RNA isolation workflow.

List of Components

Table 2. Components of EVery100B-1, EVery EV RNA Isolation Kit

Components	Qty/Volume	Storage Temperature
Lysis buffer	25 ml	RT
Lysis additive	3 ml	RT
Glycogen	100 µL	-20˚C
Wash solution	18 ml	RT
Elution solution	1 ml	RT
RNA 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 ≥8000 x g (≥10,000 rpm for table top microcentrifuge).
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. 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 30 µL of **Elution solution** to the column and let it stand for 2 minutes. Centrifuge for 1 minute to elute.
11. To maximize the recovery of the RNA add the eluate collected from step 10 back on the column, let it stand for 2 minutes. Centrifuge for 1 minute to elute.
12. Exosomal RNA is now ready for downstream applications.

Example Data and Applications

EVery RNA captures Everything

To demonstrate the ability of the EVery EV RNA isolation Kit 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 EVery 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.

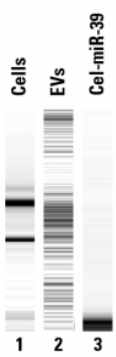

Figure 2. EVery EV RNA Isolation Kit captures EVerything.

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

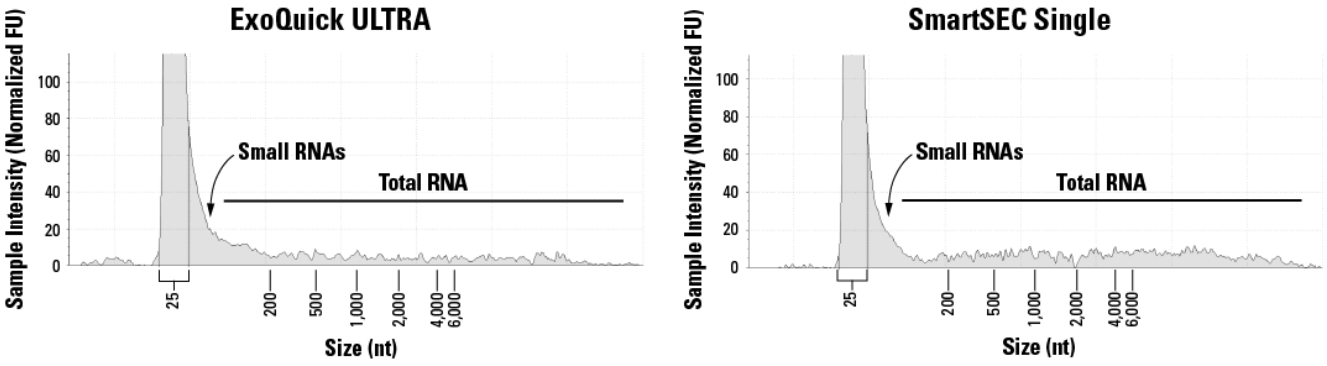

Figure 3. EVery EV RNA Isolation Kit is compatible with EVs isolated using ExoQuick Ultra and SmartSEC Single.

EVery EV RNA Isolation Kit delivers similar amounts of RNA as phenol-based methods

To demonstrate the excellent RNA yields and robust cDNA synthesis obtained with EVery family of products, we isolated EVs from 250 µL of serum using SmartSEC Single, spiked in 0.1 pmol of Cel-miR-39, and used both EVery EV RNA Isolation Kit and a phenol-based kit to isolate RNA. The isolated RNA was reverse transcribed using EVery cDNA Synthesis Kit and the copy number of Cel-miR-39 measured (Figure 4). EVery EV RNA Isolation Kit delivered similar levels of Cel-miR-39 as the phenol-based method.

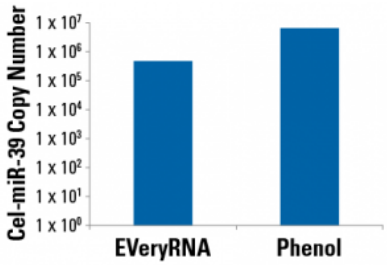

Figure 4. EVery EV RNA Isolation is EVery bit as good as phenol.

EVery EV RNA Isolation Kit efficiently isolates mRNA

We used the EVery EV RNA Isolation Kit and EVeryRNA cDNA Synthesis 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.

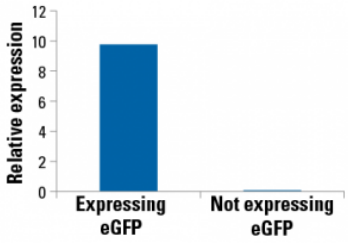

Figure 5. EVery efficiently isolates longer RNAs like mRNA

miRNA isolated with EVery100B-1 and converted to cDNA using EVery200B-1 can be used for miRNA profiling

We isolated EVs from 250 µL of normal or breast cancer patient serum using SmartSEC Single and used EVery EV RNA Isolation Kit and EVery cDNA Synthesis Kit to isolate and reverse transcribe EV RNAs for miRNA profiling using EVery miRNome Profiler for Human Serum and Plasma (EVery500B-1). Distinct miRNA expression profiles of breast cancer patient serum compared to normal serum are shown in Figure 6. Differential expression of selected breast cancer associated miRNA markers were further confirmed by qPCR (Figure 7).

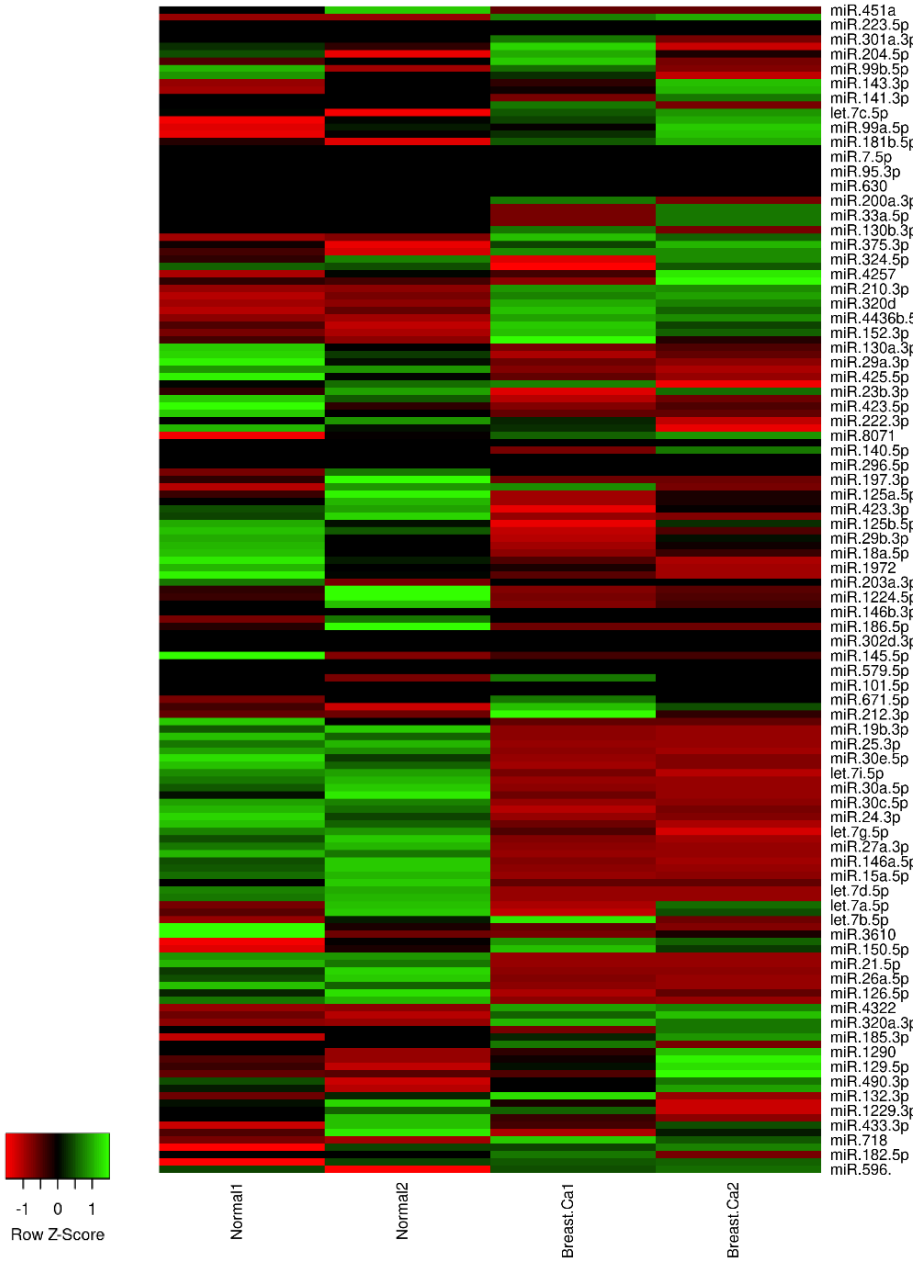

Figure 6. EVery family of products generate high-quality cDNA suitable for miRNA profiling

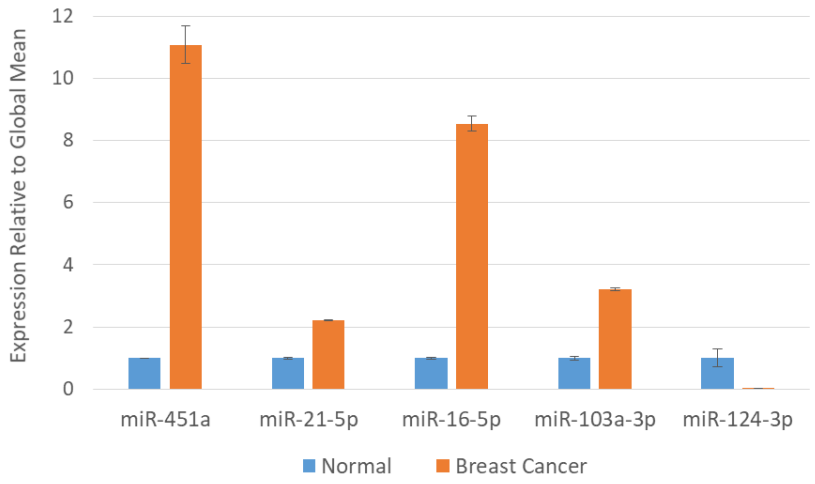

Figure 7. Differential expression of selected miRNAs in normal vs. breast cancer serum confirmed by qPCR. Data normalized with global mean. Expression of miR-451a, miR-21-5p, miR-16-5p and miR-103a are significantly upregulated, while expression of the tumor suppressor miR-124-3p is significantly downregulated in breast cancer serum compared to normal serum.

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

Table 3. Successful RNA-seq with EVery-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

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