EVeryRNA™ EV RNA Purification System with SmartSEC Single

Cat # EVery110SS-1

User Manual

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

Version 2
3/17/2022

Contents

Product Description

Get enhanced SEC-based EV isolation with SmartSEC Single and total EV RNA purification with EVeryRNA in one convenient kit. Discover more when you capture total EV RNA

Combine the contaminant-trapping enhanced SEC-based purification of SmartSEC Single 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
- Ensure delivery of highly pure RNA by using the included DNaseI
- Isolate RNA from EVs for a full range of downstream applications, such as RNA-seq and miRNA profiling
- Maximize productivity with SmartSEC Single bundled with EVeryRNA

The EVeryRNA EV RNA Purification System with SmartSEC Single comes with sufficient reagents to perform 10 EV isolation reactions followed by 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)

Work Flow: Maximize productivity with SmartSEC Single and EVeryRNA

Step 1: Isolate EVs with SmartSEC Single

The SmartSEC Single workflow is fast and easy. Simply apply 100 – 250 µL of cleared serum or plasma with additional column buffer or up to 4 mL of other biofluids directly to the pre-washed column, incubate, and centrifuge to elute the EVs.

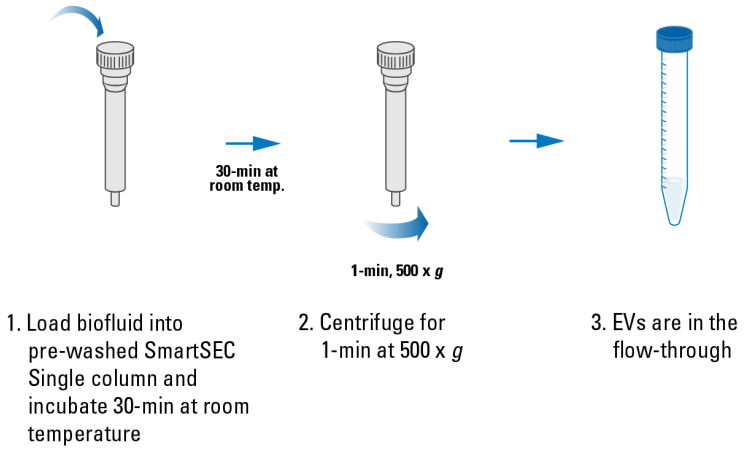


Figure 1. The SmartSEC Single workflow

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).

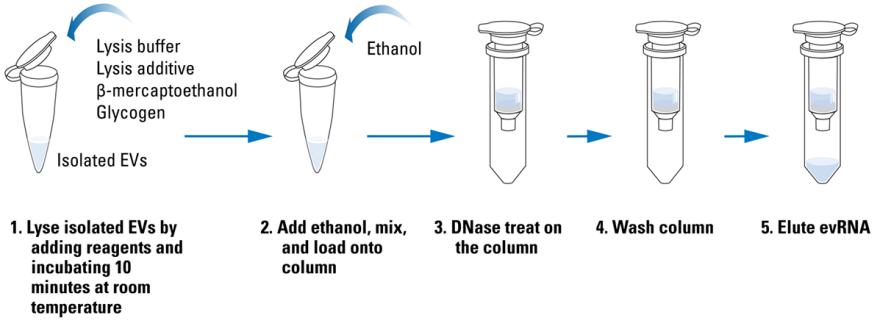


Figure 2. The quick and easy EVeryRNA EV RNA purification workflow.

List of Components

Table 2. Components of EVery106SS-1, EVeryRNA™ EV RNA Purification System

Components	Qty/Volume	Storage Temperature
SmartSEC Single column	10	RT
Column buffer	50 ml	RT
Lysis buffer	12.5 ml	RT
Lysis additive	1.5 ml	RT
Glycogen	50 µl	-20˚C
Wash solution	18 ml	RT
Elution solution	0.5 ml	RT
Micro spin column	10	RT
Collection tubes	10	RT
Elution Tubes	10	RT
RNase free DNase I	100 µl	-20˚C
Enzyme buffer	1 ml	RT

Note: The table above is for 10 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: Isolate EVs with SmartSEC Single

The protocol is optimized for 100 µl – 250 µl of serum/plasma samples.

Since serum/plasma samples contain large amounts of blood related proteins (Albumin, IgG, globulins and etc.), we don't recommend to apply more than 250 µl of the sample, particular if your downstream application is mass spectrometry analysis or TEM imaging.

If your biofluid is not serum or plasma you can consider increasing the volume of the starting material up to 4 mL (for example CSF, Urine).

Sample preparation:

1. Collect the biofluid and centrifuge at 3,000 × g for 15 minutes to remove cellular debris.
2. To remove large vesicles differential centrifugation step at 10,000-12,000 × g for 15 minutes is optional.

EVs isolation:

1. Take **SmartSEC Single column** (resin contained in storage buffer) and remove the cap and the bottom closure.

   ! **CAUTION:** save the cap and the bottom closure for later steps.
2. Place the column in an empty **Collection tube**.
3. Centrifuge at 500 xg for 30 sec to remove storage buffer.
4. Add 1 mL of **Column buffer** to the column.
5. Centrifuge at 500 xg for 30 sec to wash the beads.
6. Discard the collection tube.
7. The column is ready to your sample.
8. Place **bottom closure** back on the column and apply your sample on top of the resin bed.
9. Add 2 x volume of PBS to the column (for example: for sample volume 250 µl applied, add 500 µl of 1xPBS or column buffer) and close the column with the cap.
10. Incubate at RT for 20 -30 min with rotation.
11. Have a sample collection tube of your choice ready for EVs collection (not provided).
12. Loosen the cap half way and remove the bottom closure.

    ! **CAUTION:** if the bottom closure is removed first, the sample will start leaking out from the column. In addition, we strongly recommend keeping the cap half way inserted into the column due to the residual liquid that might still be present in the cap.
13. Place the column in the sample collection tube (step 11) and centrifuge at 500 xg for 30 sec to collect EVs.
14. Store your EVs for immediate use at +4°C up to one week. For long term storage store isolated EVs at -20°C or -80°C.

Step 2: Purify EV RNA with EVeryRNA

Before you start the protocol for exosomal RNA isolation:

1. The protocol is outlined for 700 µl) of EV sample input isolated from SmartSEC Single.
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 low RNA yield.

4. Prepare a working concentration of the **Wash Buffer** 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 of **Lysis Buffer**, 150 µl of **Lysis Additive** and 10 µl of β-mercaptoethanol (optional) to the 700 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 are expecting that RNA yield will be low.

3. After incubation add 1.85 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.

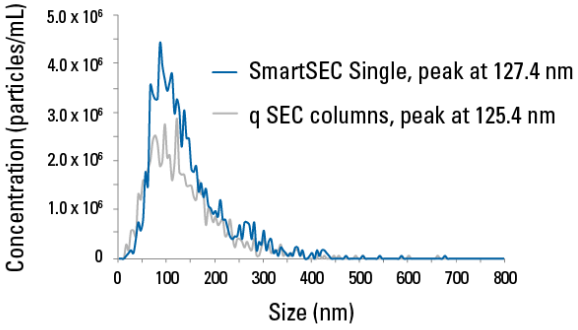
7. Apply 600 µl of **Wash solution** to the column and centrifuge for 30 seconds. Discard the flowthrough and reassemble the spin column in its collection tube.
8. Repeat step 9 one more time, for a total of two washes.

9. Centrifuge the empty column for 1 minute to completely remove any residual buffe. 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 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 to elute.
12. Exosomal RNA is now ready for downstream applications.

Example Data and Applications

SmartSEC Single performs better than competitor's q SEC technology

To understand how well SmartSEC Single performs compared to a competitor's q SEC columns, we isolated EVs from 250 µL of human serum using both SmartSEC Single and q SEC columns and analyzed 1 µg of protein equivalent from each isolation method using fluorescent nanoparticle tracking analysis (fNTA, Figure 3). EVs isolated using SmartSEC Single show a higher number of particles per mg of protein (Figure 3) than EVs isolated using the q SEC columns.



	Serum	
Isolation method	SmartSEC Single	q SEC columns
Concentration (particles/mL)	9.3×10^{10}	6.7×10^{9}
Yield (particles)	6.9×10^{10}	1.0×10^{10}
Purity (particles/mg)	23.3×10^{10}	11.0×10^{9}

Figure 3. fNTA show that SmartSEC Single delivers higher yields of cleaner EVs than a competitor's q SEC column.

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 4, 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 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 4. EVeryRNA captures EVerything.

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

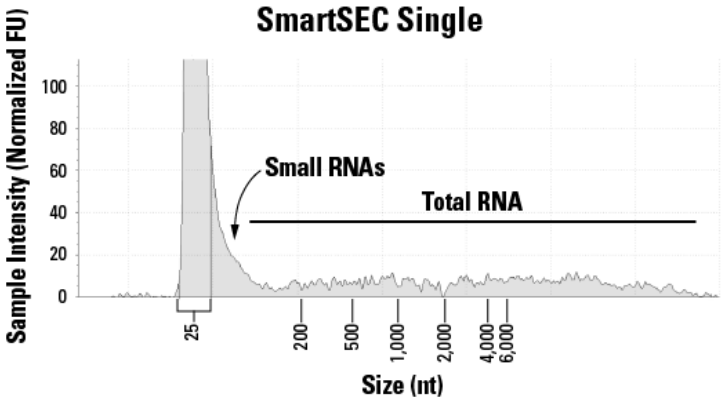


Figure 5. EVeryRNA is compatible with EVs isolated using SmartSEC Single.

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

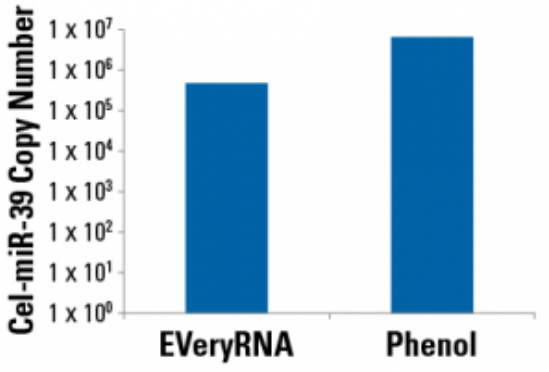


Figure 6. EVeryRNA is EVery bit as good as phenol.

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.

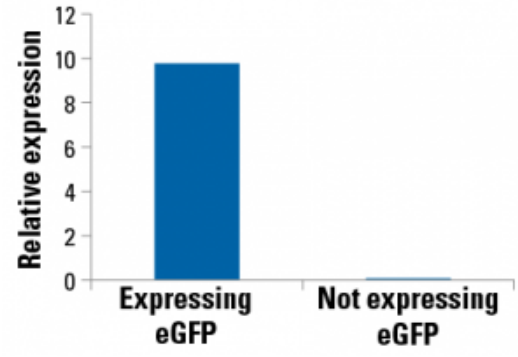


Figure 7. EVeryRNA efficiently isolates longer RNAs like mRNA

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).

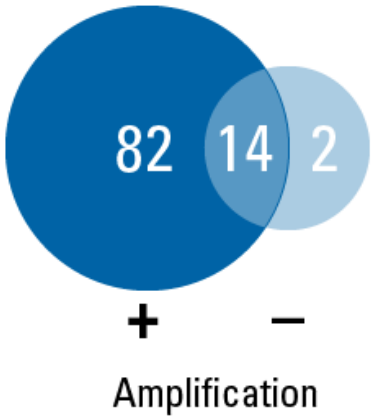


Figure 8. EVeryRNA generates high-quality RNA suitable for miRNA profiling both with and without amplification.

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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