EVery cDNA Synthesis Kit

Cat # EVery200B-1

User Manual

Storage:
Store at -20°C upon receipt

Version 1
8/25/2022

Contents

Product Description

Get your EV RNA ready for downstream analysis with this quick and easy cDNA synthesis kit optimized for use with the EVery EV RNA Isolation Kit.

- Get quick (<2 hrs) and easy cDNA synthesis optimized for use with EVery EV RNA Isolation Kit
- Use with most downstream applications such as PCR, qPCR and miRNA profiling
- Includes sufficient Universal reverse primer for all 8 profiles from EVery miRNome Profiler for Human Serum and Plasma, EVery500B-1

Optimized for use with EVery EV RNA Isolation Kit, EVery cDNA Synthesis Kit provides high quality exosomal cDNA for your downstream applications such as PCR, qPCR, miRNA profiling, and RNA-seq. The kit generates cDNA through poly(A) tailing of exosomal RNA followed by reverse transcription with MMLV Reverse Transcriptase. This method uses the template-switching oligonucleotide property of MMLV RT to generate cDNA molecules with two adaptor primers from both 5'- and 3'- ends (Figure 1), the expression of which can be detected by qPCR using EVery miRNome Universal Reverse Primer included in the kit. EVery cDNA Synthesis Kit comes with sufficient reagents to perform 20 reactions.

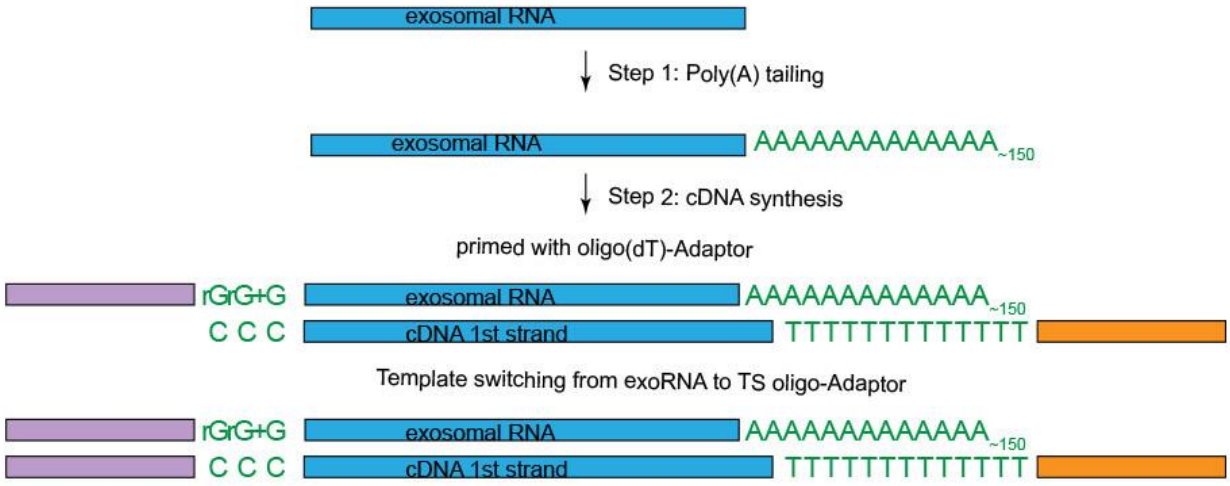

Figure 1. EVery cDNA synthesis workflow.

List of Components

Table 2. Components of EVery cDNA Synthesis Kit, EVery200B-1

Components	Qty/Volume	Storage Temperature
5X Poly(A) tailing reaction buffer	40 µL	-20˚C
10 mM ATP	20 µL	-20˚C
Poly(A) tailing enzyme	10 µL	-20˚C
RNase inhibitor	20 µL	-20˚C
RNase free water	400 µL	-20˚C
Oligo(dT)-Adaptor	40 µL	-20˚C
5X First strand buffer	100 µL	-20˚C
DTT	40 µL	-20˚C
dNTPs	40ul	-20˚C
TS oligo-Adaptor	20 µL	-20˚C
First strand synthesis enzyme	40 µL	-20˚C
Universal Reverse Primer	3 vials of 500 µL	-20˚C

Additional Required and Optional Equipment Not Included in Kit

1. PCR tubes
2. Thermal cycler
3. EVery miRNA Spike-In Kit, EVery600B-1 can be used to determine the efficiency of cDNA synthesis.

Protocol

Poly(A) tailing reaction and cDNA synthesis:

1. Set up the following reaction:

Component	Quantity
5X Poly(A) tailing reaction buffer	2 µL
10 mM ATP	1 µL
Poly(A) tailing enzyme	0.5 µL
RNase inhibitor	0.25 µL
EVs RNA	X µL
RNase free water	Up to 10 µL

2. Gently mix the reaction and centrifuge briefly.
3. Incubate the tube at 37^0C for 20 minutes.
4. Add **2 µL of Oligo(dT)-Adaptor** to prime the cDNA.
5. Incubate at 72^0C for 3 minutes and then, 2 minutes on ice.
6. Add the following components for the first strand synthesis reaction:

Component	Quantity
5X First strand buffer	5 µL
dNTPs	2 µL
DTT	2 µL
TS oligo- Adaptor	1 µL
First strand synthesis enzyme	2 µL
RNase free water	Up to 25 µL

7. Place the tube in the thermal cycler with a heated lid, preheated to 42^0C. Run the following program:

   42°C – 90 min
   70°C – 10min
   40°C – forever

Example Data and Applications

Get robust cDNA synthesis with EVery cDNA Synthesis Kit

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 2). EVery EV RNA Isolation Kit delivered similar levels of Cel-miR-39 as the phenol-based method.

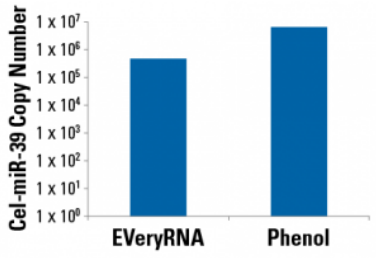

Figure 2. EVery is every bit as good as phenol.

EVery efficiently isolates mRNA

We used EVery EV RNA Isolation Kit and EVery cDNA Synthesis Kit to isolate mRNA and synthesize cDNA from cells overexpressing eGFP (Figure 3). Robust levels of eGFP mRNA are recovered and converted to cDNA when cells are overexpressing eGFP.

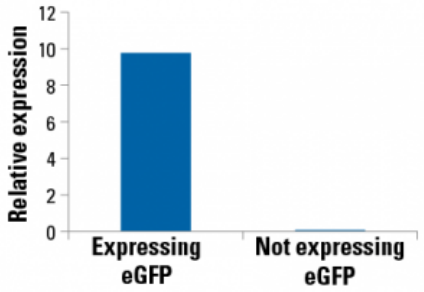

Figure 3. EVery efficiently isolates longer RNAs like mRNA

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 4. Differential expression of selected breast cancer associated miRNA markers were further confirmed by qPCR (Figure 5).

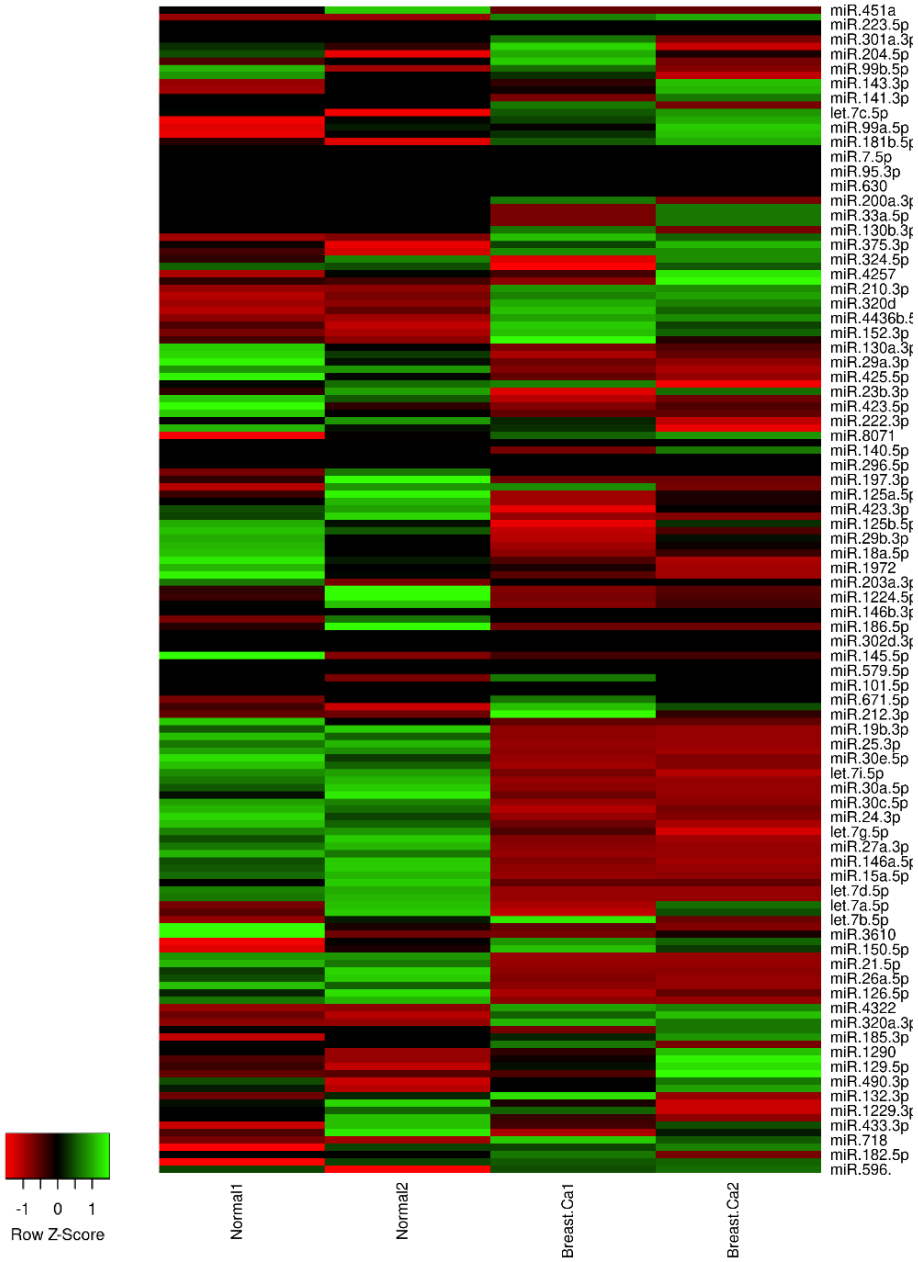

Figure 4. EVery family of products generate high-quality cDNA suitable for miRNA profiling

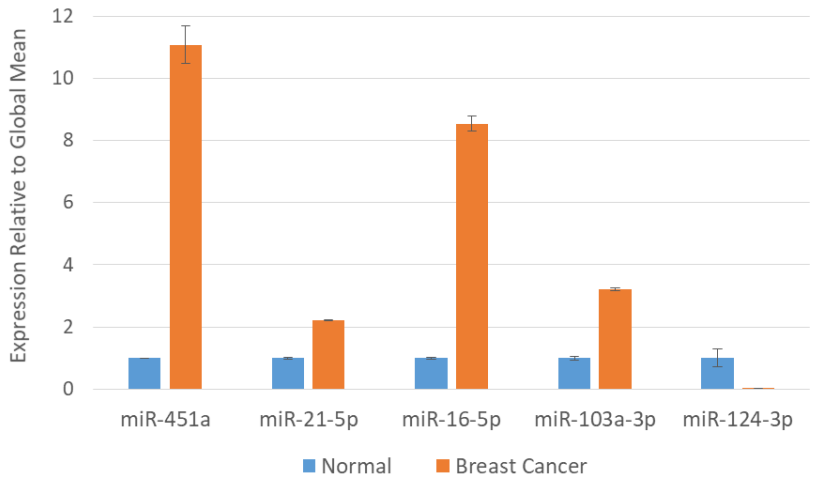

Figure 5. Differential expression of selected miRNAs in normal vs. breast cancer serum confirmed by qPCR. Data normalized with global mean. Expression of miR-451a, miR-21-5p, miR-16-5p and miR-103a are significantly upregulated, while expression of the tumor suppressor miR-124-3p is significantly downregulated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

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