EVery miRNome Profiler for Human Serum and Plasma

Cat # EVery500B-1

User Manual

Please see individual components for storage conditions

Version 1
8/24/2022

Contents

Product Description

Streamline your studies of miRNAs with this EVery miRNome Profiler for Human Serum and Plasma that contains the primers dispensed and lyophilized in each well in an amount sufficient for one 10 µl reaction per well. Each profiler plate includes primers for 182 miRNA (in duplicates), carefully selected based on literature and in-house NGS data of miRNAs in serum/plasma exosomes, as well as some candidate reference miRNAs (hsa-miR-103a-3p, hsa-miR-191-5p, hsa-miR-16-5p, hsa-miR-423-3p and hsa-let-7a-5p), from miR-451a and miR-23a to monitor hemolysis, 5 synthesized RNA spike-ins as control for protocol and IPC (inter plate control) for plate uniformity. All miRNA assays are based on miRBase entries.

From just 250 ul of serum or plasma one can assay for differential expression of preselected 182 exosomal miRNAs (in duplicates) between normal and diseased samples. This can be significant for biomarker discovery and validation, opening up potential for diagnosis and treatment monitoring.

EVery miRNome Profiler for Human Serum and Plasma has been extensively validated to work equally well with various upstream EV isolation (ExoQuick, ExoQuick-Ultra and SmartSEC) and EV RNA isolation products (RA808A-1 and EVery100B-1) from SBI's portfolio. Once you have already isolated EV RNA from your samples you may simply start with EVery200B-1, EVery cDNA Synthesis Kit to make the cDNA template that is ready to go into qPCR with EVery miRNome Profiler for Human Serum and Plasma

- Screen 182 pre-selected exosomal miRNAs (in duplicates), carefully chosen based on literature and in-house NGS data of miRNAs in serum/plasma exosomes
- Identify miRNA biomarkers and expression pattern signatures
- Compatible with various EV and EV-RNA isolation methods upstream
- Use as little as nanogram amounts of starting EV RNA
- Conduct high-throughput screens of clinical samples
- Contains inbuilt controls for sample quality, monitoring hemolysis, isolation efficiency and interplate variation.
- Primers dispensed and lyophilized in each well in an amount sufficient for one 10 µl reaction per well.

List of Components

Components	Quantity	Storage Temperature
EVery miRNome Profiler plate (384-well)	4 plates (8 panels)	-20°C
Optical adhesive film cover	4	15-25°C

The product to order consists of 4x one 384-well PCR plate, compatible with most real-time PCR instruments (Table 1) and with the following features:

- Each 384-well plate with two replicates of 182 publication-validated miRNA primers in human serum/plasma exosomes.
- 6 candidate reference miRNAs
- 5 synthesized RNA spike-ins as built-in controls for protocol
- 3 inter-plate-controls for plate uniformity

Table 1. Instrument Compatibility of PCR plate

Real-time PCR Instrument
ABI 3130xl Genetic Analyzer, 3500xL Dx Genetic Analyzer, 3730xl DNA Analyzer, 7900HT System, GeneAmp 9700, QuantStudio™, Veriti Dx Thermal Cycler, Veriti Thermal Cycler, ViiA™ 7 Dx System, ViiA™ 7 System, QuantStudio™ Dx, 3130 Genetic Analyzer, 3500 Genetic Analyzer, 3730 DNA Analyzer, 3500xL Genetic Analyzer, 3500 Dx Genetic Analyzer
Bio-Rad CFX 384
Roche LightCycler 480
Qiagen QIAquant 384

Storage

The kits are shipped on blue ice, dry ice or room temperature. Plates are stored at -20C and should be used within 6 months from date of receipt. Optical adhesive film covers are stored at room temperature.

Additional Material Required

- 2X SYBR Green Master Mix
- Nuclease-free water
- Real-time PCR thermal cycler
- Universal reverse primer compatible with adaptor used for cDNA synthesis
- EVery cDNA Synthesis Kit (EVery200B-1)

Protocol

The EVery miRNome Profiler for Human Serum and Plasma (EVery500B-1) are produced in ready-to-use format, with the primers dispensed and lyophilized in each well in an amount sufficient for one 10 µl reaction per well.

Important points before starting

- This protocol is designed to determine the expression profile for miRNA from human serum and plasma EVs.
- This protocol is for real-time PCR analysis using universal reverse primer compatible with the adaptor used for custom cDNA synthesis.
- We recommend using EVery miRNA Spike-in Kit (EVery600B-1) as sample preparation quality controls.
- This protocol is for use with SYBR green based PCR master mix on any real-time PCR cycler.

Things to do before starting

- Take out the EVery miRNome Profiler plate and briefly centrifuge to collect contents at the bottom of wells.

Quantitative, Real-time PCR Using the EVery miRNome Profiler

1. Prepare a reaction mix according to Table 2.

Table 2. Reaction setup for EVery miRnome PCR Profiler

Component	192 assays	384 assays
2X SYBR Green* Master Mix	1000 µL	2000 µL
Universal reverse primer	60 µL	120 µL
cDNA template (Undiluted)	8 µL (≥ 20 ng**)	16 µL (≥ 40 ng**)
Nuclease-free water	932 µL	1864 µL
Total	**2000 µL**	**4000 µL**

*** SBI has tested and recommends the following SYBR Green Master mix: PowerUp™ SYBR™ Green Master Mix from Applied Biosystems™; Maxima SYBR Green/ROX qPCR Master Mix (2X) from Thermo Scientific™.**

**** Suggested cDNA quantity determined by NanoDrop.**

2. Vortex the reaction mix thoroughly and dispense 10 µl per well into the EVery miRNome Profiler plate.

3. Seal the plate with the optical adhesive film cover, and carefully vortex it to dissolve the primers. Briefly centrifuge the plate and wait 5 min for the primers to dissolve completely in the reaction mix.

4. Program the real-time PCR cycler according to the guidelines as detailed for your specific real-time PCR instrument. The cycling conditions in Table 3 were used by SBI on an ABI QuantStudio 6K Flex Real-time PCR System, and can also be applied to any other real-time PCR system.

Table 3. PCR Cycling conditions

Step	Temperature	Duration	Cycles
Pre-treatment (optional)	50°C	2 min	hold
Initial denaturation	95°C	10 min	hold
Denaturation	95°C	15 sec	40
Annealing/Extension	60°C	1 min	
Melting curve analysis			

Analysis and Data Interpretation

The complete list and layout of the profiler plate can be found in the spreadsheet:

https://www.systembio.com/wp/wp-content/uploads/2022/08/08232022-EVery-miRNome-EVery500B-1_Human_Serum_384-Layout.xlsx

Data normalization

In qPCR experiments, it is important to include stably expressed transcripts for proper normalization of data. Because miRNAs are very short RNA species, they may behave very differently during the isolation and reverse transcription process than the longer RNA transcripts. In general, normalization may be performed using the global mean of all expressed miRNAs. Alternatively, normalization can be performed with some stably expressed endogenous miRNAs, depending on the origin of your samples. Candidate reference miRNAs for serum and plasma are hsa-miR-103a-3p, hsa-miR-191-5p, hsa-miR-451a, and hsa-miR-23a (all included in the EVery miRNome Profiler).

Quality assessment

The EVery miRNome Profiler contains matching primers for detecting RNA spike-ins from EVery miRNA Spike-in Kit (EVery600B-1). Adding known RNA spike-ins Spkn1, Spkn2 and Spkn3 to the samples prior to RNA isolation provide a way to check for RNA isolation efficiency. Adding the spike-ins Spkn4 and cel-miR-39-3p during cDNA synthesis monitors the potential presence of inhibitors in the cDNA synthesis. For detailed information see EVery miRNA Spike-in Kit (EVery600B-1) user manual. EVery miRNome Profiler also contains a set of unique, independent inter-plate-controls (IPC) to check for overall success of the qPCR reactions and the plate uniformity. Examples of spike-in and IPC assessment data can be found in Figure 1.

Monitoring hemolysis

Hemolysis in serum and plasma samples can cause contamination to the overall miRNA profile from cell-derived miRNA. The expression of the red blood cell specific miR-451a increases in the case of hemolysis, independent of the serum stable miR-23a. Therefore, data from miR-451a and miR-23a can be used to monitor hemolysis. Briefly, a ΔCt(miR-23a – miR-451a) lower than 5 in human serum or plasma represents non-hemolyzed samples, while a ΔCt(miR-23a – miR-451a) close to or higher than 7 indicates an increased possibility of hemolysis. For example, the Ct values of miR-23a and miR-451a for serum sample used in Figure 1 are 31.76 and 28.54, respectively. Thus, ΔCt(miR-23a – miR-451a) for the sample is 3.22, reflecting that no significant hemolysis is present. Note, that not all miRNAs are affected by hemolysis. Large variations in the degree of hemolysis across samples within a project may introduce noise to the data interpretation and removal of outlier samples should be considered.

Example Data and Applications

Expression of spike-ins and reference miRNAs

EVery miRNome Profiler for Human Serum or Plasma has been extensively validated to work equally well with various upstream EV isolation (ExoQuick, ExoQuick-Ultra and SmartSEC) and EV RNA isolation products (RA808A-1 and EVery100B-1) from SBI's portfolio (Figure 1). EVery cDNA Synthesis Kit is used to make the cDNA template. Expression of the 6 candidate reference miRNAs were analyzed (Figure 2).

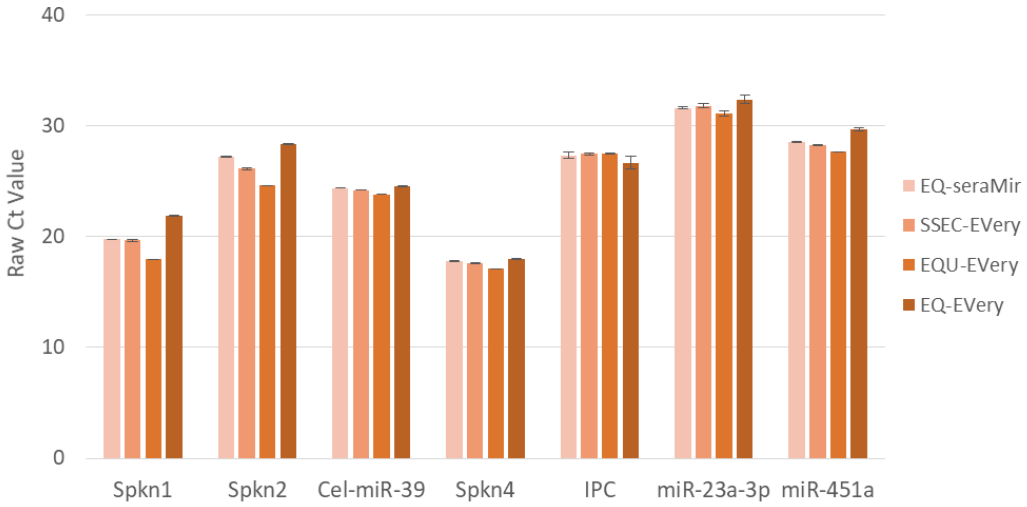

Figure 1. Spike-ins and reference RNAs reflect comparable efficiency of RNA isolation from EVs isolated using different EV isolation products from SBI. The synthetic RNA spike-in mixture was added during sample preparation stepwise according to EVery miRNA Spike-in Kit (EVery600B-1) user manual. miR-23a and miR-451 are reference miRNAs stably expressed in serum. Error bars are standard errors from 3 replicate isolations. All SBI EV RNA isolation products show robust results in assessing sample EV miRNA expression.

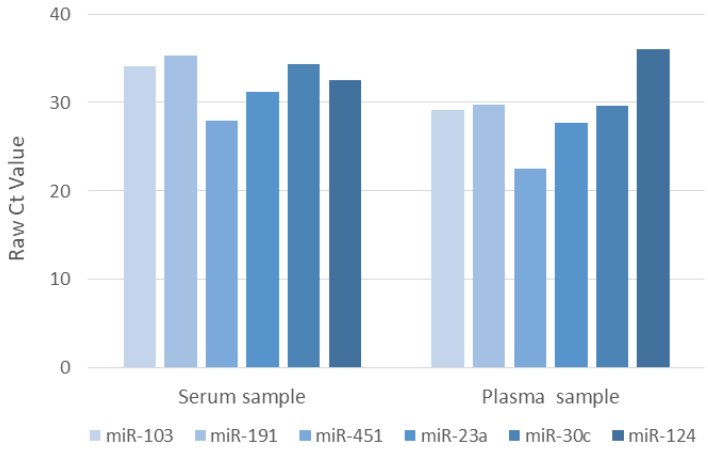

Figure 2. Expression of 6 candidate reference miRNAs in human serum and plasma samples. miR-103 and miR-191 are well expressed in most tissues; miR-23a and miR-451 are stably expressed in serum/plasma. miR-30c and miR-124 are widely found in urine and CSF, respectively.

miRNA expression profiles of normal vs. disease sample

Starting with 250 µL of serum sample each from normal or breast cancer patient, EV RNA was isolated using SmartSEC Single and EVery EV RNA Isolation Kit, followed by cDNA synthesized with the EVery cDNA Synthesis Kit. Expression of the exosomal miRNA in normal vs. breast cancer serum samples were analyzed by qPCR using the EVery miRNome Profiler (Figure 3). Differential expression of selected breast cancer associated miRNA markers were confirmed by qPCR (Figure 4).

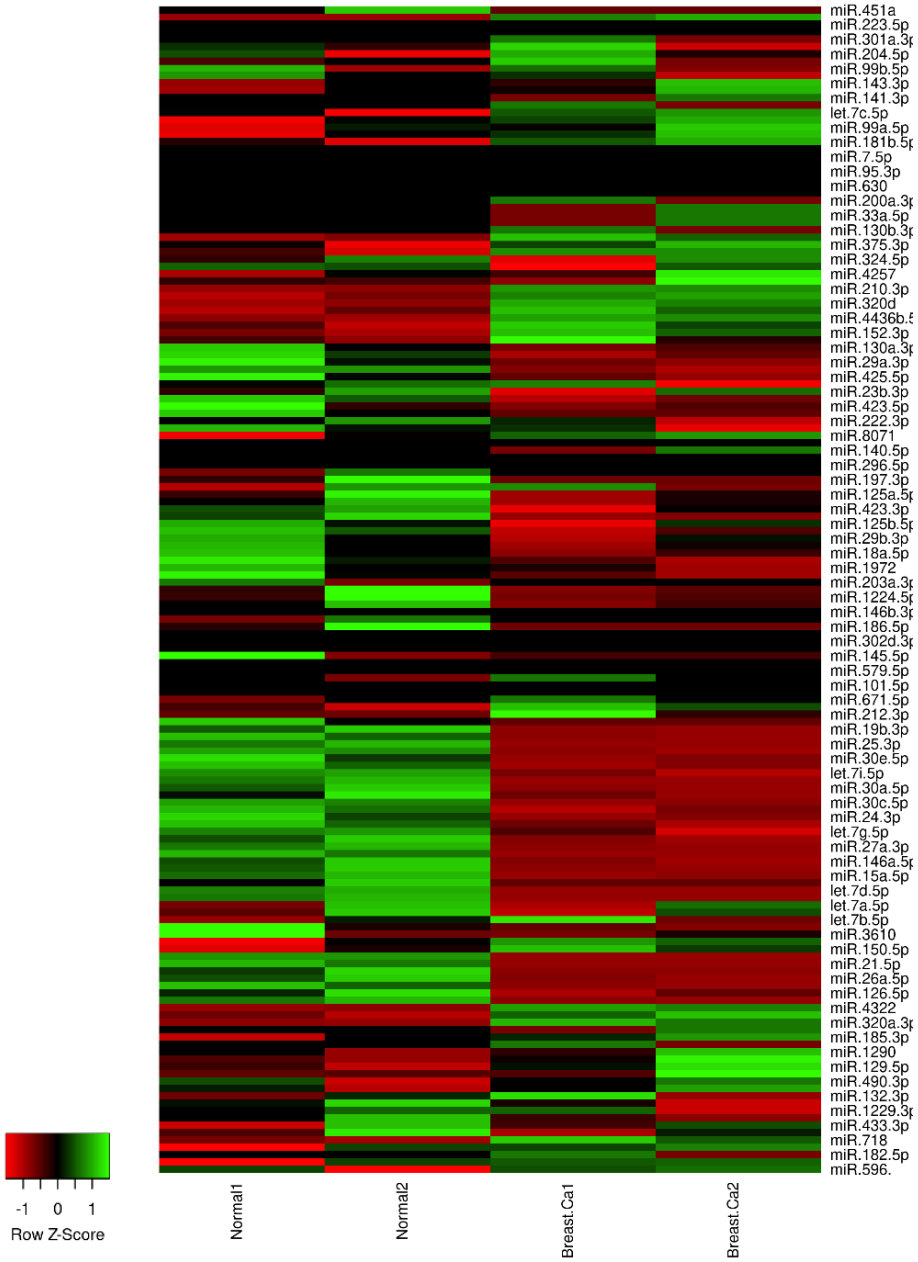

Figure 3. Heat map of normal vs. breast cancer serum miRNA expression profiles. Representative data normalized to global mean of each sample. Breast cancer patient serum showed distinct miRNA expression profiles compared to normal serum.

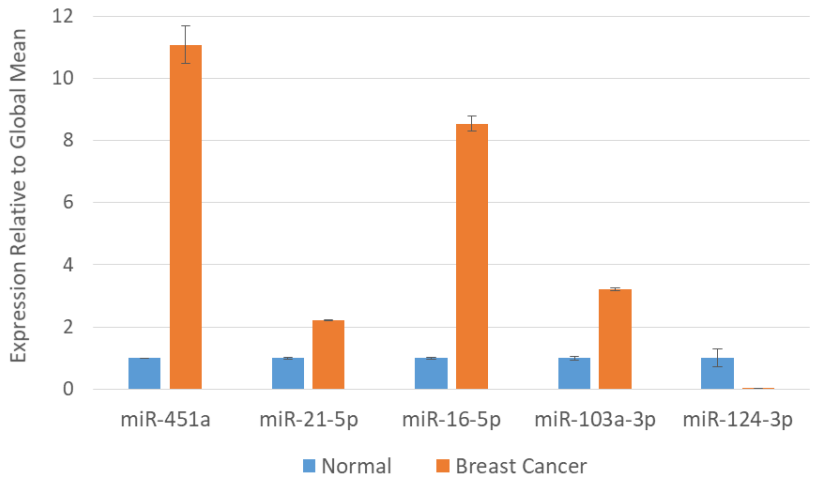

Figure 4. EVery miRNome Profiler identifies differential expression of selected miRNAs in normal vs. breast cancer serum. Data normalized with global mean. Expression of miR-451a, miR-21-5p, miR-16-5p and miR-103a are significantly upregulated, while expression of the tumor suppressor miR-124-3p is significantly downregulated in breast cancer serum compared to normal serum.

Next Steps and Related Products

Application	Product	Catalog Number
Exosome/EV Isolation		
High-purity, high-yield SEC-based isolation from a range of biofluids		
High-throughput SEC-based isolation from serum and plasma, 96-well format	**SmartSEC™ HT**	**SSEC096A-1**
Single format SEC-based isolation, validated for human serum, plasma, and CSF	**SmartSEC™ Single**	**SSEC200A-1**
High purity, polymer-based EV isolation		
Isolation from serum and plasma	**ExoQuick® ULTRA**	**EQULTRA-20A-1**
General purpose, polymer-based EV isolation		
Isolation from serum and plasma	**ExoQuick®**	**EXOQ20A-1**
RNA extraction from Exosomes		
Obtain high yields of total exosome/EV RNA, including small RNAs	**EVery EV RNA Isolation Kit**	**EVery100B-1**
Flexible & efficient RNA extraction from exosomes	**SeraMir Exosome RNA Column Purification Kit**	**RA808A-1**
cDNA synthesis from EV RNA		
Quick and easy cDNA synthesis optimized for use with the EVery EV RNA Purification System	**EVery cDNA Synthesis Kit**	**EVery200B-1**
Quality control for EV miRNA profiling		
Synthetic small RNA controls for your RNA isolation and cDNA synthesis steps	**EVery miRNA Spike-in Kit**	**EVery600B-1**

Technical Support

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

For additional information or technical assistance, please call or email us at:

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Licensing and Warranty Statement

Limited Use License

Use of the EVery miRNome Profiler for Human Serum and Plasma (*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.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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