EVery miRNA Spike-in Kit

Cat # EVery600B-1

User Manual

Please see individual components for storage conditions

Version 1
8/24/2022

Contents

Product Description

Quality and integrity of RNA as well as efficiency of isolation methods is critical to maintain the representation of any transcriptome. Because miRNAs are very short RNA species, they may behave very differently during the isolation and reverse transcription process than the longer RNA transcripts. Therefore, it is very important to have controls that resemble endogenous miRNAs closely. Synthetic RNAs that resemble the size of endogenous miRNAs but do not share any homology with any identified miRNAs are ideal spike-ins. Optimized mix of such spike-ins offers a control for endogenous miRNAs of low, medium and high abundance levels reflecting closely the fidelity or impact of any RNA isolation methods. The RNA Spike-ins Spkn1, Spkn2 and Spkn3 are at a 100-fold concentration difference, therefore, there should be ~6.6 Ct difference between the serial dilutions of spike-ins. The concentration of Spkn1 corresponds to very abundant miRNAs, and the concentration of Spkn3 corresponds to weakly expressed miRNAs and might not always be detectable. Undetectable Spkn3 could be an indication that miRNAs expressed at low levels were lost during isolation. A separate Spike-in mix (Spkn4 and cel-miR-39-3p) added later during cDNA synthesis reports on the efficiency of reverse transcription and PCR amplification that may be affected by inhibitors.

- Separate Spike-in mixes for RNA isolation and cDNA synthesis steps
- Suitable for miRNA from any source or method of isolation
- Cover high, medium and low abundance miRNAs
- Forward primer supplied for qPCR

List of Components

Components	Volume	Storage Temperature
Spkn1, Spkn2, Spkn3 RNA Spike-in Mix	100 µL	-80°C
Spkn4, cel-miR-39-3p RNA Spike-in Mix	100 µL	-80°C
Spkn1 Forward Primer	30 µL	-80°C
Spkn2 Forward Primer	30 µL	-80°C
Spkn3 Forward Primer	30 µL	-80°C
Spkn4 Forward Primer	30 µL	-80°C
cel-miR-39-3p Forward Primer	30 µl	-80°C

General Information

The product contains two sets of RNA Spike-in Mix, and 5 matching forward primers for detecting the spike-ins by qPCR:

- Spkn1, Spkn2, Spkn3 RNA Spike-in Mix for use in RNA isolation
- Spkn4, cel-miR-39-3p RNA Spike-in Mix for use in cDNA synthesis
- The RNA spike-ins and all primers are premixed and resuspended in nuclease free water.

Storage

The kits are shipped on dry ice and should be stored at -80°C. The product should be used within 6 months from date of receipt.

It is strongly recommended to store the RNA Spike-in mix in aliquots to avoid repeated freeze-thaw cycles. Freeze-thaw will result in degradation of the RNA Spike-in mix.

Follow general precautions for handling of RNA. Use RNase free tips, tubes, and reagents in RNase free environment when handling EVery miRNA Spike-in Kits.

Protocol: Using the Spkn1, Spkn2, Spkn3 RNA Spike-in Mix in RNA isolation

1. Thaw the RNA Spike-in mix (Spkn1, Spkn2 and Spkn3) on ice. Mix by vortexing and briefly centrifuge. Store in aliquots at -80°C to avoid repeated freeze-thaw cycles.
2. Use the lysis buffer of your choice to lyse your samples.
3. Add **1 µl** of the "Spkn1, Spkn2, Spkn3 RNA Spike-in mix" **per RNA prep** to the sample lysate.

   Important note: Do not add the RNA spike-in mix directly to your samples. It may be rapidly degraded.
4. Proceed with the RNA isolation protocol as usual.
5. Expression of the RNA Spike-ins (Spkn1, Spkn2 and Spkn3) can be detected by qPCR using the supplied primers at 0.3 ul per 10 ul of qPCR reaction, or using the EVery miRNome Profiler (EVery500B-1).

Protocol: Using the Spkn4, cel-miR-39-3p RNA Spike-in Mix in cDNA Synthesis

1. Thaw the RNA Spike-in mix (Spkn4 and cel-miR-39-3p) on ice. Mix by vortexing and briefly centrifuge. Store in aliquots at -80°C to avoid repeated freeze-thaw cycles.
2. When setting up reverse transcription reactions, add **1 µl** of the "Spkn4, cel-miR-39-3p RNA Spike-in mix" **per 20 µl** of RT reaction.

   Important note: Adjust the amount of Spkn4 and cel-miR-39-3p RNA spike-in mix to the volume of RT reaction.
3. Expression of the RNA Spinke-ins (Spkn4 and cel-miR-39-3p) can be detected by qPCR using the supplied primers at 0.3 ul per 10 ul of qPCR reaction, or using the EVery miRNome Profiler (EVery500B-1).

Analysis and Interpretation of Data

The RNA spike-ins Spkn1, Spkn2 and Spkn3 are at a 100-fold concentration difference, therefore, there should be ~6.6 Ct difference between the serial dilutions of spike-ins. Examples of spike-in assessment data can be found in Figure 1. The concentration of Spkn1 corresponds to very abundant miRNAs, and the concentration of Spkn3 corresponds to weakly expressed miRNAs and might not always be detectable. Undetectable Spkn3 could be an indication that miRNAs expressed at low levels were lost during isolation. In this case, use more RNA for the cDNA synthesis or improve the yield of the RNA isolation. (Data validated using the EVery EV RNA Isolation Kit and the EVery cDNA Synthesis Kit for EV RNA isolation and first strand cDNA synthesis.)

Overview of issues and conclusions using serum/plasma EV samples

For serum/plasma EV samples, a fixed amount of the RNA spike-ins were added per isolation, and a fixed volume of isolated RNA was used for cDNA synthesis. Therefore, the main factors affecting the amplification signals of the RNA isolation control (Spkn1, Spkn2 and Spkn3) are isolation efficiency, cDNA synthesis efficiency and amplification efficiency. The main factors affecting the amplification signals of the cDNA synthesis control (Spkn4 and el-miR-39-3p) are cDNA synthesis efficiency and amplification efficiency. Here is a quick guidelines to interpret the Ct values of the controls:

Control type	Ct values	Conclusion	Action to consider
RNA isolation controls (Spkn1, Spkn2, Spkn3)	Consistent values across all samples (< 2-3 Ct); ΔCt = 5-7 between spike-ins	RNA isolation OK	Include in study
	Increased Ct, but Spkn4 & cel-miR-39-3p look good	• Poor RNA isolation • RNase contamination • Degradation of RNA Spike-in mix	Re-isolate RNA or exclude from study
cDNA synthesis controls (Spkn4, cel-miR-39-3p)	Consistent values across all samples (< 1-2 Ct)	cDNA synthesis OK	Include in study
	Increased Ct	• Presence of inhibitors • Degradation of RNA Spike-in mix	Re-isolate RNA or exclude from study

Example Data and Applications

Expression of spike-in miRNAs in serum and plasma EV samples

To demonstrate the excellent EV RNA yields and robust cDNA synthesis, we isolated EVs from 250 µL of serum or plasma using SmartSEC Single, spiked in with our synthetic EVery miRNA Spike-in mix, and used the EVery EV RNA Isolation Kit and the EVery cDNA Synthesis Kit. Examples of RNA spike-ins isolated with serum and plasma samples are shown in Figure 1.

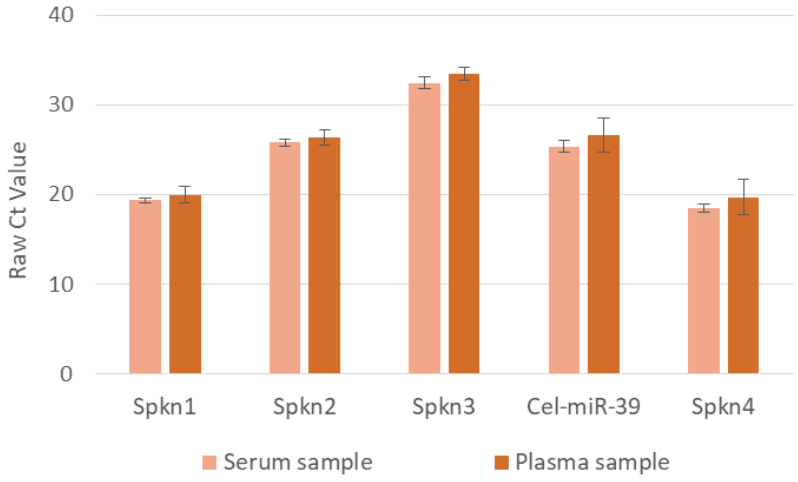

Figure 1. EVery miRNA Spike-in Kit reflects successful miRNA isolation. EV RNA from human serum and plasma samples were isolated with EVery family of products. The synthetic RNA spike-in mixture was added during sample preparation stepwise according to the RNA spike-in kit instruction manual. The RNA spike-in yields can be monitored with Spkn1, 2, and 3; the potential presence of inhibitors can be monitored with cel-miR-39 and Spkn4. The stepwise difference in Ct values between the RNA isolation spike-ins (Spkn1, 2, 3) was in the expected range of 5-7. Error bars are standard errors from 3 replicate isolations.

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:

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)
Fax: (650) 968-2277
E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

Licensing and Warranty Statement

Limited Use License

Use of EVery miRNA Spike-in Kit (*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.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll Free (888) 266-5066
Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com