Quick-Start Protocol September 2017

miRCURY® LNA® RT Kit

The miRCURY LNA RT Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. It is recommended that the RNA spike-in be stored in aliquots at –30 to –15°C after resuspension to avoid repeated freeze-thaw cycles.

Further information

- *miRCURY LNA miRNA PCR Handbook*: **www.qiagen.com/HB-2431**
- *miRCURY LNA miRNA PCR – Exosomes, Serum/Plasma and Other Biofluid Samples Handbook*: **www.qiagen.com/HB-2439**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Set up all reactions on ice to minimize the risk of RNA degradation.
- dNTPs are already included in the kit components. Do not add additional dNTPs.
- The RNA Spike-in Kit is an internal extraction and amplification control that can be used to test RNA isolation, reverse transcription and amplification, and is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors. For more information, please refer to the *RNA Spike-in Kit, for RT Quick-Start Protocol*.
- The RT primer is included in the 5× miRCURY RT Reaction Buffer.
- The 10x miRCURY RT Enzyme Mix contains both the poly(A) polymerase and the reverse transcriptase.
- It is recommended to set up the reactions in 200 µl PCR tubes and to use a PCR cycler for the incubation steps.

- The temperature steps can be conveniently set up using the cycling protocol described in Table 2.

1. Thaw template RNA and 5× miRCURY RT Reaction Buffer on ice. Thaw RNase-free water at room temperature (15–25°C). Immediately before use, remove the 10x miRCURY RT Enzyme from the freezer. Mix each solution by flicking the tubes. Centrifuge briefly to collect residual liquid from the sides of the tubes and then keep on ice.
2. Resuspend the UniSp6 RNA spike-in by adding 80 µl nuclease-free water to the tube. Mix by vortexing and spin down. Leave for 20–30 min on ice to completely dissolve the RNA spike-in. Mix by vortexing and spin down. Store in aliquots at –30 to –15°C.
3. Adjust each template RNA to 5 ng/µl. For RNA isolated from serum/plasma, calculate the amount of RNA equivalent to that isolated from 16 µl sample to be used for a 20 µl-RT reaction (i.e., for a sample isolated from 200 µl plasma and eluted in 50 µl, use 4 µl eluate).
4. Prepare the reverse transcription master mix on ice according to Table 1. Mix and then keep on ice.

   Note: If setting up more than one reaction, prepare a master mix with a volume 10% greater than required for the total number of reactions. Distribute the appropriate volume of master mix into individual tubes, followed by each RNA sample.

Table 1. Reverse transcription reaction components

Component	miRCURY LNA miRNA PCR Assay	miRCURY LNA miRNome PCR Panels – human, mouse, and rat (Panel I+II)	miRCURY LNA Focus PCR Panel – serum/Plasma	miRCURY LNA Focus PCR Panel – cancer	Pick-&-Mix < 100 miRNA analyzed per sample	Pick-&-Mix > 100 miRNA analyzed per sample
5× miRCURY RT Reaction Buffer	2 µl	8 µl	4 µl	2 µl	2 µl	4 µl
RNase-free water	4.5 µl	18 µl	9 µl	4.5 µl	4.5 µl	9 µl
10x miRCURY RT Enzyme Mix	1 µl	4 µl	2 µl	1 µl	1 µl	2 µl
Synthetic RNA spike-ins, optional	0.5 µl	2 µl	1 µl	0.5 µl	0.5 µl	1 µl
Template RNA (5 ng/µl)	2 µl	8 µl	4 µl *	2 µl	2 µl	4 µl
Total reaction volume	**10 µl**	**40 µl**	**20 µl**	**10 µl**	**10 µl**	**20 µl**

* The equivalent of 16 µl of the original serum/plasma sample is used per 20 µl reverse transcription reaction.

5. Incubate for 60 min at 42°C.
6. Incubate for 5 min at 95°C to inactivate the Reverse Transcriptase Enzyme.

Table 2. RT temperature protocol

Step	Time	Temperature	Comment
Reverse transcription step	60 min	42°C	
Inactivation of reaction	5 min	95°C	
Storage	Forever	4°C	

7. Immediately cool to 4°C.

   Note: If not used immediately, store at 4°C. For long-term storage, store the reverse transcription reactions at –30 to –15°C.

8. Place the reverse transcription reactions on ice and proceed directly with real-time PCR. Recommendations for dilutions in sterile water are available in the respective handbooks, depending on downstream application.

   Note: The miRCURY LNA SYBR® Green PCR Kit is recommended for real-time PCR. For detailed information on use of the RNA Spike-in Kit and interpretation of real-time PCR results, please refer to the *RNA Spike-in Kit, for RT Quick-Start Protocol*.

Scan QR code for *miRCURY LNA miRNA PCR Handbook*.

Scan QR code for *miRCURY LNA miRNA PCR – Exosomes, Serum/Plasma and Other Biofluid Samples Handbook*.