Quick-Start Protocol September 2017

RNA Spike-in Kit, for RT

The RNA Spike-in Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. It is recommended to store the RNA spike-ins in aliquots at –30 to –15°C after re-suspension to avoid repeated freeze-thaw cycles.

Further information

- *RNA Spike-in Kit, for RT Handbook:* **www.qiagen.com/HB-2433**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This kit contains synthetic RNA spike-in templates for use with the miRCURY LNA® miRNA PCR System, to monitor RNA isolation, reverse transcription and PCR amplification, and is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors. Inhibitors such as phenol, ethanol, sodium dodecyl sulfate (SDS) or ethylene diaminetetraacetic acid (EDTA) may remain from the lysis and purification steps of the RNA isolation procedure.
- Three of the RNA spike-in templates (UniSp2, UniSp4, UniSp5) are provided pre-mixed in one vial, each at a different concentration of 100-fold increments. This mix is intended as an RNA isolation control.
- A second vial contains a synthetic version of a *C. elegans* miRNA (cel-miR-39-3p). The cel-miR-39-3p RNA spike-in template is meant to be used in combination with the UniSp6 RNA spike-in template provided with the miRCURY LNA RT Kit (cat. no. 339340). This mix is intended as a cDNA synthesis control.

- In all miRCURY pre-defined miRNA PCR Panel Plates and Custom miRNA PCR Panels, a minimum of 3 UniSp3 assays with templates are provided. These can be used both as inter-plate calibrators as well as PCR amplification controls.
- The spike-in templates are dessicated and must be resuspended before use.

1. Spin down the vials before opening.
2. Resuspend the spike-in mix by adding 80 µl RNase-free water to the vial. Leave for 20 min on ice to completely dissolve the RNA pellet.

   Important: For the RT controls, first resuspend the UniSp6 RNA spike-in by adding 80 µl nuclease-free water to the vial as described below. Resuspend the cel-miR-39-3p RNA spike-in by adding 80 µl of the resuspended UniSp6 RNA spike-in to the vial.
3. Mix by vortexing and spin down. Store in aliquots at –30 to –15°C to avoid repeated freeze-thaw cycles.
4. For use as an RNA isolation control, prior to starting the RNA isolation/purification, add 1 µl synthetic UniSp2, UniSp4, UniSp5 RNA spike-in mix per RNA prep to the lysis buffer.

   Important: The spike-in RNA template must be mixed with the lysis buffer before mixing with the sample – it may be rapidly degraded if added directly to the sample.
5. For use as reverse transcription control prior to the RT reaction, add 1 µl synthetic spike-in mix per 20 µl cDNA synthesis.

   Note: If the cel-miR-39-3p RNA spike-in is not to be used, follow steps described in the *miRCURY LNA miRNA PCR Kit Handbook*.

Data analysis and interpretation of results

1. The synthetic RNA spike-ins should not be used for normalization. Normalization should always be performed with endogenous microRNA, either as verified stably expressed reference microRNAs or as a global mean of all expressed microRNAs (where applicable).

2. The UniSp2, UniSp4, UniSp5 RNA spike-ins are provided at different concentrations in 100-fold increments. Therefore, UniSp2 should amplify at the level of very abundant microRNAs, UniSp4 should amplify approximately 6.6 cycles later than UniSp2, and UniSp5 should amplify approximately 6.6 cycles later than UniSp4. The concentration of UniSp5 corresponds to weakly expressed microRNAs and might not always be detectable.
3. If UniSp5 is not detected, this could mean that microRNAs expressed at low levels were lost during isolation. In this case, it is recommended to use more RNA for the cDNA preparation or improve the yield of the sample preparation.
4. If all samples give comparable values for each RNA isolation control, this could suggest that all isolations were performed with similar efficiencies. However, if one or more samples give higher C_q values for the isolation controls, this could suggest that there was a problem in one of the steps of the RNA isolation procedure. If the isolation controls and endogenous reference genes are affected in a few samples, but the cDNA synthesis controls are stable across all samples, it is likely that the affected RNA samples were isolated with a lower efficiency than the remaining samples. In this case, consider re-purifying RNA from these samples, or alternatively, exclude them from the study.
5. If the isolation controls, cDNA synthesis controls and endogenous reference genes are all affected by elevated C_q values in a few samples, this could indicate the presence of RT or qPCR inhibitors in these samples. Consider excluding these samples from the study, or alternatively, consider re-purifying RNA from these samples.
6. If the endogenous controls are affected by high C_q values, while none of the RNA spike-ins are affected, this would indicate that the samples in question had a lower miRNA content to begin with. In this case, consider excluding these samples from the study.
7. For more information please consult the relevant handbook.

Scan QR code for handbook.