Quick-Start Protocol February 2018

# QIAseq™ miRNA Library QC Spike-Ins

The QIAseq miRNA Library QC Spike-Ins (cat. no. 331535) should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer. After resuspension, store the Spike-Ins in aliquots at –80°C and avoid repeated freeze–thaw cycles. If stored under these conditions, the kit contents are stable until the date indicated on the box label.

## Further information

- *QIAseq miRNA Library QC PCR Handbook*: **www.qiagen.com/HB-2437**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- Prior to first use, resuspend the QIAseq miRNA Library QC Spike-Ins in 500 µl nuclease-free water. Vortex for 5 s and leave the suspension on ice for 30 min to dissolve. Vortex for 5 s again and centrifuge to collect contents in the bottom of the tubes.
- Thaw previously resuspended QIAseq miRNA Library QC Spike-Ins on ice, briefly centrifuge and keep on ice until frozen again.

## Recommended usage

It is recommended to add the QIAseq miRNA Library QC Spike-Ins during RNA isolation to monitor the comparability and reproducibility of the whole process from RNA isolation to sequencing. Alternatively, the spike-ins can be added directly to the RNA, prior to library preparation. However, adding the spike-ins after RNA isolation will result in loss of information on the RNA isolation, especially with regard to the small RNA fraction.

The small RNA content of samples can differ significantly from sample to sample and source to source. For more accurate ratios of spike-ins vs. endogenous small RNAs in the samples,

experimental titration of QIAseq miRNA Library QC Spike-Ins is recommended. Consider Table 1 as a guide for QIAseq miRNA Library QC Spike-In amounts.

**Table 1. Recommended usage amounts of QIAseq miRNA Library QC Spike-Ins.**

| Sample type | Approximate amount of QIAseq miRNA Library QC Spike-In to use |
|---|---|
| Serum/plasma | 1 µl per isolation from 500 µl serum/plasma* |
| Cells or tissue | 1–10 µl per isolation, corresponding to ~1–2 µl spike-ins per volume of RNA eluate to be made into a library. Note that results of RNA isolation from cells or tissue will vary depending on the isolation method and use of mechanical tissue homogenization. |
| RNA from serum/plasma | 1 µl per RNA eluate of 25–50 µl (originating from 500 µl serum/plasma)* |
| RNA from cells or tissue | 1–2 µl per 100 ng RNA to be made into a library |

* The recommendations above are adjusted to elution volumes from biofluid isolations around 20–30 µl. Input material from biofluids is 6–10 µl into library preparation.

## Evaluation of the QIAseq miRNA Library QC Spike-Ins data

The 52 QIAseq miRNA Library QC Spike-Ins added during RNA isolation are present at different concentrations and if used in the right amounts will represent miRNAs ranging from very low to very high endogenous levels, with QIAseq miRNA Library QC Spike-Ins accounting for approximately 1–3% of total reads.

QIAseq miRNA Library QC Spike-Ins are very useful for evaluating NGS performance and can be used to assess the technical reproducibility across samples and verify the linearity of the NGS reads mapped to these exogenous sequences across samples.

This protocol will provide sufficient reads derived from the QIAseq miRNA Library QC Spike-Ins for post-sequencing analysis using a standard small RNA NGS analysis pipeline. Reads should be mapped to the QIAseq miRNA Library QC Spike-In sequences (using Bowtie2 or similar mapping algorithm) and spike-in reads should be filtered out from the rest of the data. We recommend "perfect match" settings when mapping, filtering and counting QIAseq miRNA Library QC Spike-In reads in a dataset (FASTQ files). Following counting of the

QIAseq miRNA Library QC Spike-In reads, they should be normalized to the total number of reads per sample. If TPM (tags per million reads) are to be used then use the following formula. For each QIAseq miRNA Library QC Spike-In, calculate TPM based on each sample:

$$\text{TPM} = \frac{\text{\# of Spike-In reads}}{\text{Total reads}} \times 1{,}000{,}000$$

After this simple normalization to individual sample reads has been done for all spike-ins in all samples, correlation matrices should be plotted for all sample-to-sample comparisons. This is done to evaluate the sample-to-sample correlation in the sample set. Expected correlation should be $R^2$ of 0.95–0.99. If comparing day-to-day correlation, the correlation is usually weaker than within a batch of samples purified on the same day. If samples deviate from these values they could be technical outliers and should potentially be excluded from downstream analysis.

Note that a few of the QIAseq miRNA Library QC Spike-Ins are present at low concentrations to represent very low TPM counts and will therefore give low numbers of reads, especially if the sequencing depth is not high. They should be excluded from the correlation analysis. A rule of thumb would be to exclude QIAseq miRNA Library QC Spike-In data lower than 1 TPM if read depth is approximately 10 million reads per sample.

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