Quick-Start Protocol March 2016

miScript® II RT Kit

The miScript II RT Kit (cat. nos. 218160, 218161, also provided as part of the miScript PCR Starter Kit, cat. no. 218193) should be stored at –30 to –15°C upon arrival.

Further information

- *miScript PCR System Handbook*: **www.qiagen.com/HB-0235**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Total RNA, containing miRNA, should be used as the starting material. It is not necessary to enrich for small RNA. We recommend miRNeasy Kits or PAXgene® miRNA Kits for purification of total RNA including miRNA. For further information, visit **www.qiagen.com/miRNA**.
- The miScript II RT Kit includes 2 buffers: **5x miScript HiSpec Buffer** and **5x miScript HiFlex Buffer**. Only one of these buffers should be used in each reverse-transcription reaction. The correct buffer to use depends on the subsequent PCR application:
  Use 5x miScript HiSpec Buffer to prepare cDNA for subsequent mature miRNA profiling using miScript miRNA PCR Arrays or quantification of mature miRNAs only using miScript Primer Assays (see Table 1). miScript HiSpec Buffer should also be used to prepare cDNA for subsequent preamplification using the miScript PreAMP PCR Kit.
  Use 5x miScript HiFlex Buffer to prepare cDNA for subsequent real-time PCR applications that may involve quantification of mature miRNAs in parallel with precursor miRNAs, mRNAs and/or other noncoding RNAs using miScript Primer Assays, miScript Precursor Assays and/or QuantiTect® Primer Assays (see Table 1).
- **IMPORTANT: Only 5x miScript HiSpec Buffer should be used to prepare cDNA for real-time PCR with miScript miRNA PCR Arrays.**
- **IMPORTANT: Only 5x miScript HiSpec Buffer should be used to prepare cDNA for preamplification with the miScript PreAMP PCR Kit.**

- **IMPORTANT**: Do not use 5x miScript HiFlex Buffer with the miScript PreAMP PCR Kit or miScript miRNA PCR Arrays.
- This protocol is for use with up to 2 µg RNA if 5x miScript HiSpec Buffer is used, or for up to 1 µg RNA if 5x miScript HiFlex Buffer is used. If using higher RNA amounts, scale up the reaction linearly. Recommended amounts of template RNA to use in this protocol depend on the downstream PCR application and are shown in Table 1. If RNA sample is limiting (10 ng–100 ng), and the sample is intended for use with miScript miRNA PCR Arrays, we highly recommend preparing cDNA with 5x miScript HiSpec Buffer and preamplifying with the miScript PreAMP PCR Kit.

Table 1. Recommended RNA starting amounts and buffers

PCR application	Assay/array	Buffer	Recommended RNA input*
Preamplification	Any miScript Array	5x miScript HiSpec Buffer	10–100 ng per RNA sample
Pathway profiling of mature miRNA	Pathway-Focused miScript miRNA PCR Arrays	5x miScript HiSpec Buffer	125–250 ng per RNA sample†
Pathway profiling of mature miRNA (high content)	miScript miRNA HC PCR Arrays	5x miScript HiSpec Buffer	250–500 ng for one 384-well plate†
Whole miRNome profiling of mature miRNA	miRNome miScript miRNA PCR Arrays	5x miScript HiSpec Buffer	250–500 ng per 384-well plate or per 4 x 96-well plates/Rotor-Discs (the number of plates provided in a miRNome miScript miRNA PCR Array varies depending on the species of interest)†
Mature miRNA quantification only	miScript Primer Assays	5x miScript HiSpec Buffer	Depends on abundance and number of target miRNAs to be quantified; from 10 ng up to a maximum of 2 µg
Parallel real-time PCR quantification of mature miRNAs, precursor miRNAs, mRNAs and/or other noncoding RNAs	miScript Primer Assays, miScript Precursor Assays and/or QuantiTect Primer Assays	5x miScript HiFlex Buffer	Depends on abundance and number of target miRNAs to be quantified; up to a maximum of 1 µg
Precursor miRNA detection	miScript Precursor Assays	5x miScript HiFlex Buffer	Depends on abundance and number of target miRNAs to be quantified; up to a maximum of 0.5 µg

* If the RNA sample is not limiting, use the upper limit of the recommended range. For more information on sample input, refer to the *miScript PCR System Handbook* or *miScript miRNA PCR Array Handbook*.

† These recommended RNA starting amounts result in 0.5–1 ng cDNA per array well.

1. Thaw template RNA on ice. Thaw 10x miScript Nucleics Mix and either 5x miScript HiSpec Buffer or 5x miScript HiFlex Buffer at room temperature (15–25°C). Mix each solution by flicking the tubes. Centrifuge briefly to collect residual liquid from the sides of the tubes and then store on ice.
2. Prepare the reverse-transcription master mix on ice according to Table 2. Mix and then store on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA.

   IMPORTANT: **If cDNA will be used for preamplification with the miScript PreAMP PCR Kit, use a final reaction volume of 10 µl**. For next steps, refer to the *miScript PreAMP PCR Kit Quick-Start Protocol* and the *miScript PreAMP Handbook*.

   Note: miScript Reverse Transcriptase Mix should be removed from the –20°C freezer just before preparation of the master mix and placed on ice. It should be returned to the freezer immediately after use.

Table 2. Reverse-transcription reaction components

Component	Volume/reaction	Volume/reaction for later preamplification
5x miScript HiSpec Buffer or 5x miScript HiFlex Buffer*	4 µl	2 µl
10x miScript Nucleics Mix	2 µl	1 µl
RNase-free water	Variable	Variable
miScript Reverse Transcriptase Mix	2 µl	1 µl
Template RNA (added in step 3)	Variable (see Table 1)	Variable (see Table 1)
Total volume	**20 µl**	**10 µl**

* The correct buffer to use depends on the subsequent PCR application, see "Notes before starting" and Table 1.

3. Add template RNA to each tube containing reverse-transcription master mix. Mix gently, briefly centrifuge and then place on ice.
4. Incubate for 60 min at 37°C.
5. Incubate for 5 min at 95°C to inactivate miScript Reverse Transcriptase Mix and place on ice. To proceed immediately, dilute the reactions as described in Table 3, mix gently, briefly centrifuge and continue with real-time PCR or preamplification. Alternatively, to store prior to real-time PCR or preamplification, transfer undiluted to a –20°C freezer.

Table 3. cDNA dilution prior to PCR or preamplification

PCR application	Assay/array	Reaction dilution
Preamplification	Any miScript Array	Add 40 µl RNase-free water to the 10 µl reverse-transcription reaction
Pathway profiling	Pathway-Focused miScript miRNA PCR Arrays	Add 200 µl RNase-free water to each 20 µl reverse-transcription reaction
Pathway profiling (high content)	miScript miRNA HC PCR Arrays	Add 90 µl RNase-free water to the 20 µl reverse-transcription reaction
Whole miRNome profiling	miRNome miScript miRNA PCR Arrays	Dilution depends on the number of plates/Rotor-Discs: For 1 x 384-well plate or 4 x 96-well plates/Rotor-Discs: add 90 µl RNase-free water to the 20 µl reverse-transcription reaction For 2 x 384-well plate or 8 x 96-well plates/Rotor-Discs: add 200 µl RNase-free water to the 20 µl reverse-transcription reaction For 3 x 384-well plate or 12 x 96-well plates/Rotor-Discs: add 310 µl RNase-free water to the 20 µl reverse-transcription reaction For 4 x 384-well plate or 16 x 96-well plates/Rotor-Discs: add 420 µl RNase-free water to the 20 µl reverse-transcription reaction
Mature miRNA quantification	miScript Primer Assays	Depends on abundance of miRNAs of interest; ensure 50 pg–3 ng cDNA per PCR by adding at least 200 µl RNase-free water, or more if necessary, to the 20 µl reverse-transcription reaction
Parallel quantification of mature miRNAs, precursor miRNAs, mRNAs, other noncoding RNAs	miScript Primer Assays, miScript Precursor Assays and/or QuantiTect Primer Assays	Depends on abundance of RNAs of interest; for parallel detection of mature miRNA with either precursor miRNA and/or mRNA, ensure 10–20 ng cDNA per PCR; for parallel detection of mature miRNA and other noncoding RNAs, ensure 50 pg–3 ng cDNA per PCR
Precursor miRNA detection	miScript Precursor Assays	Depends on abundance of precursor miRNA of interest; ensure 10–20 ng cDNA per PCR

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