Quick-Start Protocol April 2016

miScript SYBR® Green PCR Kit used with miScript Primer Assays or miScript Precursor Assays

The miScript SYBR Green PCR Kit (cat. nos. 218073, 218075, 218076) should be stored between –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

- This protocol is for real-time PCR using nonamplified cDNA template prepared with the miScript II RT Kit. (For preamplified cDNA refer to the *miScript PreAMP Handbook*). The 20 µl reverse-transcription reaction should be prepared according to the *miScript PCR System Handbook* and diluted as shown in Table 1.
- This protocol is for real-time PCR using a miScript Primer Assay (for detection of mature miRNA or other noncoding RNA) or a miScript Precursor Assay (for detection of precursor miRNA). For a real-time PCR protocol using a QuantiTect Primer Assay (for detection of mRNA), consult the *miScript PCR System Handbook*.
- **IMPORTANT**: If detection of mature miRNA only is desired, ensure that the cDNA template has been prepared using the miScript II RT Kit with **miScript HiSpec Buffer**.
- **IMPORTANT**: If real-time PCR applications may involve quantification of mature miRNAs in parallel with precursor miRNAs, mRNAs and/or other noncoding RNAs, ensure that the cDNA template has been prepared using the miScript II RT Kit with **miScript HiFlex Buffer**.

- If using the miScript Primer Assay or miScript Precursor Assay for the first time, be sure to reconstitute it before use according to the instructions in the *miScript PCR System Handbook*.
- If using the miScript miRNA PCR Arrays, refer to the *miScript miRNA PCR Array Handbook*.
- Prior to preparing real-time PCR reaction mixes, thaw all reagents (2x QuantiTect SYBR Green PCR Master Mix, 10x miScript Universal Primer and RNase-free water) and the cDNA templates prepared using the miScript II RT Kit. Gently mix the contents in each tube and briefly centrifuge.
- Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 1. cDNA dilution prior to PCR

Application	Assay	Buffer used in RT	Reaction dilution
Mature miRNA quantification only	miScript Primer Assays	miScript HiSpec Buffer	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 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	miScript HiFlex Buffer	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	miScript HiFlex Buffer	Depends on abundance of precursor miRNA of interest; ensure 10–20 ng cDNA per PCR

1. Prepare a reaction mix according to Table 2 (if quantifying mature miRNA only or if quantifying mature miRNA and noncoding RNA) or Table 3 (if quantifying precursor miRNA).

Table 2. Reaction mix for detection of mature miRNA only or mature miRNA and other noncoding RNA

Component	Volume/reaction (384-well)	Volume/reaction (96-well)	Volume/reaction (Rotor-Disc® 100)
2x QuantiTect SYBR Green PCR Master Mix	5 µl	12.5 µl	10 µl
10x miScript Universal Primer	1 µl	2.5 µl	2 µl
10x miScript Primer Assay	1 µl	2.5 µl	2 µl
RNase-free water	Variable	Variable	Variable
Template cDNA (added at step 2)*	≤1 µl	≤2.5 µl	≤2 µl
Total volume	**10 µl**	**25 µl**	**20 µl**

* The volume of diluted cDNA should not exceed 10% of the final reaction volume. The final concentration of cDNA should be 50 pg–3 ng per reaction. Volumes refer to cDNA prepared using the miScript II RT Kit with miScript HiSpec Buffer (mature miRNA detection only) or miScript HiFlex Buffer (detection of mature miRNA and other noncoding RNA), and diluted according to Table 1.

Table 3. Reaction mix for detection of precursor miRNA

Component	Volume/reaction (384-well)	Volume/reaction (96-well)	Volume/reaction (Rotor-Disc 100)
2x QuantiTect SYBR Green PCR Master Mix	5 µl	12.5 µl	10 µl
10x miScript Precursor Assay†	1 µl	2.5 µl	2 µl
RNase-free water	Variable	Variable	Variable
Template cDNA (added at step 2)‡§	≤1 µl	≤2.5 µl	≤2 µl
Total volume	**10 µl**	**25 µl**	**20 µl**

† The miScript Precursor Assay contains both a forward and a reverse primer. **Do not add** miScript Universal Primer.

‡ The volume of diluted cDNA should not exceed 10% of the final reaction volume. The final concentration of cDNA should be 10–20 ng per reaction. Volumes refer to cDNA prepared using the miScript II RT Kit with miScript HiFlex Buffer.

§ If quantifying mature miRNA and precursor miRNA in parallel, we recommend using the higher amount of cDNA template as recommended for precursor miRNA detection for all the reactions (10–20 ng). This enables direct comparison of results.

2. Dispense template cDNA into the individual wells of the PCR plate or Rotor-Disc.
3. Mix the reaction mix thoroughly and dispense appropriate volumes into the wells containing template cDNA.
4. Carefully, tightly seal the PCR plate or Rotor-Disc with caps, film or Rotor-Disc Heat-Sealing Film.

5. Centrifuge for 1 min at 1000 x *g* at room temperature (15–25°C).

   Note: This step is not necessary for reactions set up in Rotor-Discs.

6. Program the real-time cycler according to Table 4.

   Note: Perform dissociation curve analysis of the PCR product(s) to verify their specificity and identity. Follow the instructions provided by the supplier.

7. Place the plates in the real-time cycler and start the cycling program.

Table 4. Cycling conditions

Step	Time	Temperature	Additional comments
Initial activation step	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling:			
Denaturation	15 s	94°C	
Annealing	30 s	55°C	
Extension*	30 s	70°C	Perform fluorescence data collection.
Cycle number	40 cycles		Cycle number depends on the amount of template cDNA and abundance of the target.

* Due to software requirements, the fluorescence detection step must be at least 30 s with the ABI PRISM® 7000 or 34 s with the Applied Biosystems® 7300 and 7500.

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For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.