Quick-Start Protocol April 2016

# miScript® SYBR® Green PCR Kit used with miScript miRNA PCR Arrays

The miScript SYBR Green PCR Kit (cat. nos. 218073, 218075, 218076) should be stored at –30 to –15°C upon arrival.

## Further information

- *miScript miRNA PCR Array Handbook*: **www.qiagen.com/HB-0903**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- This protocol is for real-time PCR using cDNA prepared with the miScript II RT Kit (using miScript HiSpec Buffer) and preamplified cDNA. The reverse-transcription reaction should be diluted as shown in Table 1.
- **IMPORTANT: Only cDNA prepared with miScript HiSpec Buffer should be used for real-time PCR with miScript miRNA PCR Arrays**.
- 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 (and optionally the miScript PreAMP PCR Kit). Gently mix the contents in each tube and briefly centrifuge.
- The format of the miScript miRNA PCR Array is indicated by the last letter of the catalog number. The formats and the real-time cyclers that are supported are detailed in the *miScript miRNA PCR Array Handbook*.

**Table 1. cDNA dilution prior to PCR**

| Application | Array | Reaction dilution |
|---|---|---|
| Preamplified cDNA | Any miScript Array | Refer to *miScript PreAMP Handbook* |
| 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 each 20 µl reverse-transcription reaction |
| Whole miRNome profiling | miRNome miScript miRNA PCR Arrays | Dilution depends on the number of plates/Rotor-Discs:<br>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<br>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<br>3 x 384-well plate or 12 x 96-well plates/Rotor-Discs:<br>add 310 µl RNase-free water to the 20 µl reverse-transcription reaction<br>4 x 384-well plate or 16 x 96-well plates/Rotor-Discs:<br>add 420 µl RNase-free water to the 20 µl reverse-transcription reaction |

1. Prepare a reaction mix according to Table 2 (for Pathway-Focused miScript miRNA PCR Arrays) or Table 3 (for miRNome miScript miRNA PCR Arrays or miScript miRNA HC PCR Arrays), depending on your chosen miScript miRNA PCR Array.

**Table 2. Reaction mix for Pathway-Focused miScript miRNA PCR Arrays**

| Array format:<br>Component | 384-well (4 x 96)*<br>Formats E, G | 96-well<br>Formats A, C, D, F | Rotor-Disc® 100<br>Format R |
|---|---|---|---|
| 2x QuantiTect SYBR Green PCR Master Mix | 550 µl | 1375 µl | 1100 µl |
| 10x miScript Universal Primer | 110 µl | 275 µl | 220 µl |
| RNase-free water | 340 µl | 1000 µl | 780 µl |
| Template cDNA† | 100 µl | 100 µl | 100 µl |
| **Total volume** | **1100 µl** | **2750 µl** | **2200 µl** |

* Volumes shown are sufficient for one cDNA template. In total, 4 cDNA templates can be analyzed on one 384-well (4 x 96) Pathway-Focused miScript miRNA PCR Array.

† Provides 0.5–1 ng cDNA per well.

**Table 3. Reaction mix for miRNome miScript miRNA PCR Arrays* and miScript miRNA HC PCR Arrays†**

| Array format:<br>Component | 384-well<br>Formats E, G | 96-well<br>Formats A, C, D, F | Rotor-Disc 100<br>Format R |
|---|---|---|---|
| 2x QuantiTect SYBR Green PCR Master Mix | 2050 µl | 1375 µl | 1100 µl |
| 10x miScript Universal Primer | 410 µl | 275 µl | 220 µl |
| RNase-free water | 1540 µl | 1075 µl | 855 µl |
| Template cDNA‡ | 100 µl | 25 µl | 25 µl |
| **Total volume** | **4100 µl** | **2750 µl** | **2200 µl** |

* Volumes are for a single plate or Rotor-Disc associated with a whole miRNome set. Scale up volumes according to the number of plates/Rotor-Discs to be run.

† For miScript miRNA HC PCR Arrays, use the volumes shown for 384-well, Formats E, G.

‡ Provides 0.5–1 ng cDNA per well.

2. Carefully remove the miScript miRNA PCR Array from its sealed bag.

   **Optional for 96-well and 384-well array formats**: If the reaction mix is in a tube, transfer to a loading reservoir, such as the RT² PCR Array Loading Reservoir (cat. no. 338162).

3. Add reaction mix to each well of the miScript miRNA PCR Array as follows:

   For 384-well miScript miRNA PCR Array: add 10 µl per well.

   For 96-well miScript miRNA PCR Array: add 25 µl per well.

   For Rotor-Disc miScript miRNA PCR Array: add 20 µl per well.

4. Carefully, tightly seal the miScript miRNA PCR Array with Optical Thin-Wall 8-Cap Strips (Formats A and D), Optical Adhesive Film (Formats C, E, F and G) or Rotor-Disc Heat-Sealing Film (Format R).

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

   **Note**: This step is not necessary for Format R miScript miRNA PCR Arrays in Rotor-Discs.

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

**Note**: Perform dissociation (melting) curve analysis of the PCR product(s) to verify their specificity and identity. Dissociation curve analysis is an analysis step built into the software of real-time cyclers. Follow the instructions provided by the supplier.

**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. |

* For Bio-Rad® models CFX96™ and CFX384™: adjust the ramp rate to 1°C/s.

† For Eppendorf® Mastercycler® ep realplex models 2, 2S, 4 and 4S: for the Silver Thermoblock, adjust the ramp rate to 26%; for the Aluminum Thermoblock, adjust the ramp rate to 35%.

‡ If using a Roche® LightCycler® 480, adjust the ramp rate to 1°C/s.

§ 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.

¶ If using a Roche LightCycler 480, use 45 cycles.

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

Scan QR code for handbook.

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