miScript® PreAMP PCR Kit and miScript PreAMP Primer Mixes

The miScript PreAMP PCR Kit (cat. nos. 331451, 331452) and miScript PreAMP Primer Mixes should be stored at –20°C upon arrival. For more information, please refer to the *miScript PreAMP Handbook*, which can be found at www.qiagen.com/handbooks. For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- **IMPORTANT: Only cDNA prepared using the miScript II RT Kit with miScript HiSpec Buffer should be used as starting material for preamplification with this protocol.**
- **IMPORTANT**: We recommend performing a 10 µl reverse-transcription reaction prior to preamplification. Dilute the 10 µl reaction in 40 µl RNase-free water. Use exactly 5 µl of this diluted cDNA for preamplification as indicated in the protocol. Do not use any more or less than 5 µl. (If a reverse-transcription reaction has already been performed using a 20 µl reaction volume, dilute the 20 µl reaction in 80 µl RNase-free water and use exactly 5 µl diluted cDNA for preamplification.)
- The preamplification must start with an initial incubation step of 15 minutes at 95°C to activate HotStarTaq® DNA Polymerase.
- **IMPORTANT**: miScript PreAMP miRNome Primer Mixes may be provided in multiple tubes. In these cases, set up separate preamplification reactions and pool once the reactions are completed.
- For preamplification using a mixture of miScript Primer Assays instead of a miScript PreAMP Primer Mix, refer to the *miScript PreAMP Handbook*.

1. Prepare the preamplification master mix at room temperature according to Table 1. Mix and then store on ice.

   Note: 5x miScript PreAMP Buffer and HotStarTaq DNA Polymerase should be removed from the –20°C freezer just before preparation of the master mix and placed on ice. They should be returned to the freezer immediately after use. ▶

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Table 1. Preamplification reaction components

Component	Volume/reaction
5x miScript PreAMP Buffer	5 µl
HotStarTaq DNA Polymerase	2 µl
miScript PreAMP Primer Mix	5 µl
RNase-free water	7 µl
miScript PreAMP Universal Primer	1 µl
Template cDNA (added in step 2)	5 µl
Total volume	25 µl

2. Add template cDNA to each tube containing preamplification master mix. Mix gently, briefly centrifuge, and then place on ice.

3. Program the thermal cycler according to either Table 2 (for 96-plex preamplification reaction) or Table 3 (for 384-plex preamplification reaction).

   A 96-plex preamplification reaction amplifies 96 cDNA targets and is used with a miScript PreAMP Pathway Primer Mix or with a miScript PreAMP Custom Primer Mix (up to 96 assays) for subsequent miRNA profiling with a Pathway-Focused or Custom miScript miRNA PCR Array.

   A 384-plex preamplification reaction amplifies 384 cDNA targets and is used with a miScript PreAMP miRNome Primer Mix, miScript PreAMP Pathway HC Primer Mix, or miScript PreAMP Custom Primer Mix (96–384 assays) for subsequent miRNA profiling with a miRNome or Custom miScript miRNA PCR Array or a miScript miRNA HC PCR Array.

Table 2. Cycling conditions for 96-plex preamplification

Step	Time	Temperature
Initial activation step HotStarTaq DNA Polymerase is activated by this heating step.	15 min	95°C
2-step cycling:		
Denaturation	30 s	94°C
Annealing/extension	3 min	60°C
Cycle number	12 cycles	

Table 3. Cycling conditions for 384-plex preamplification

Step	Time	Temperature
Initial activation step HotStarTaq DNA Polymerase is activated by this heating step.	15 min	95°C
3-step cycling:		
Denaturation	30 s	94°C
Annealing	60 s	55°C
Extension	60 s	70°C
Cycle number	2 cycles	
2-step cycling:		
Denaturation	30 s	94°C
Annealing	3 min	60°C
Cycle number	10 cycles	

4. Place the preamplification reaction in the real-time cycler and start the run.

5. After the run has finished, dilute the preamplified cDNA in RNase-free water according to the following recommendations. Gently mix and then store on ice.

   Dilution factor for Pathway-Focused Arrays and Custom Arrays (up to 96 assays) = ng input cDNA x 20-fold/ng

   Dilution factor for miRNome Arrays, Custom Arrays (96 to 384 assays), and HC Arrays = ng input cDNA x 5-fold/ng

   Example of dilution for Pathway-Focused Array: If 10 ng total RNA was used in the 10 µl reverse-transcription reaction that was subsequently diluted in 40 µl RNase free water, and 5 µl diluted cDNA was used in the preamplification reaction, this results in 1 ng cDNA used as starting material for the preamplification reaction. According to the formula:

   Dilution factor = 1 ng input cDNA x 20-fold/ng = 20-fold

   For a 20-fold dilution, dilute the 25 µl preamplification reaction in 475 µl RNase free water. ▶

IMPORTANT: The minimum dilution factor for preamplified cDNA is 20-fold for Pathway-Focused Arrays and Custom Arrays (up to 96 assays) and 5-fold for miRNome Arrays, Custom Arrays (96 to 384 assays), and HC Arrays. Therefore if less than 1 ng input cDNA was used for preamplification, we recommend performing a 20-fold or 5-fold dilution, depending on the array type.

Note: If the input cDNA amount is not known, we recommend using miR-16 miScript Primer Assay or SNORD95 miScript Primer Assay or the miScript miRNA QC PCR Array to determine the optimal dilution factor. See the *miScript PreAMP Handbook* for more details.

Note: The real-time PCR reaction mix composition compensates for the differing dilutions (20-fold or 5-fold), ensuring the same amount of template preamplified cDNA in real-time PCR miRNA quantification.

IMPORTANT: For miScript PreAMP miRNome Primer Mixes provided in multiple tubes, separate preamplification reactions were performed. Pool the preamplification reactions prior to dilution.

6. Proceed with real-time PCR according the *miScript miRNA PCR Array Handbook*.

   Note: For further details and a protocol for control experiments using the miScript Primer Assays provided in the miScript PreAMP PCR Kit, see the *miScript PreAMP Handbook*.

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