November 2018

miScript® Microfluidics Handbook

miScript II RT Kit
miScript Microfluidics PreAMP Kit
miScript Microfluidics PCR Kit
miScript PreAMP Primer Mix
miScript miRNA PCR Arrays
miScript Primer Assays
miScript Primer Assay 96 Plates

For real-time profiling of miRNAs using the Fluidigm® BioMark™ System

Sample to Insight

QIAGEN

Contents

Kit Contents

miScript Microfluidics PreAMP Kit **Catalog no.** **Number of standard reactions**	**(96)** **331455** **96**
5x miScript PreAMP Buffer	2 x 300 µl
HotStarTaq® DNA Polymerase (2 U/µl)	2 x 120 µl
miScript PreAMP Universal Primer (10 µM)	2 x 60 µl
Side Reaction Reducer	2 x 96 µl

miScript Microfluidics PCR Kit **Catalog no.** **Number of 96.96 Dynamic Array™ IFCs**	**(5)** **331431** **5**
Microfluidics qPCR Master Mix	1.65 ml
miScript Microfluidics Universal Primer (40 µM)	5 x 1.4 ml

miScript II RT Kit	**(12)**	**(50)**
Catalog no.	**218160**	**218161**
Number of standard reactions	**12**	**50**
miScript Reverse Transcriptase Mix	24 µl	100 µl
10x miScript Nucleics Mix	50 µl	200 µl
5x miScript HiSpec Buffer	100 µl	400 µl
5x miScript HiFlex Buffer	100 µl	400 µl
RNase-Free Water	1.9 ml	1.9 ml

miScript PreAMP Pathway Primer Mix	**(96)**
Catalog no.	**Varies**
Number of samples	**96**
miScript PreAMP Pathway Primer Mix	530 µl

miScript PreAMP miRNome Primer Mix	**(96)**
Catalog no.	**Varies**
Number of samples	**96**
miScript PreAMP miRNome Primer Mix	530 µl per tube*

* miScript PreAMP miRNome Primer Mix for a miRNome miScript miRNA PCR Array may be provided in more than 1 tube. In these cases, separate preamplification reactions must be performed, and the preamplified cDNA pooled prior to real-time PCR (see Appendix B, page 37).

miScript PreAMP Custom Primer Mix	**(192)**
Catalog no.	**Varies**
Number of samples	**192**
miScript PreAMP Custom Primer Mix	1060 µl

miScript miRNA PCR Array, Format M	
Catalog no.	**Varies**
Single-use miRNome or biology-focused panels of miRNA primer assays ready for use with the Fluidigm BioMark System.	2

miScript Primer Assay 96 Plate **Catalog no.** **Number of assays per plate**	 **218540** **24-96**
Customer-configured miScript Primer Assays in 96-well plates. Content may be chosen by the customer at **www.qiagen.com/GeneGlobe**. Two synthesis scales are available.	1

miScript Primer Assay **Catalog no.**	 **Varies***
Lyophilized miScript Primer Assay (contains one miRNA-specific primer) in tube	1

* Visit **www.qiagen.com/GeneGlobe** to search for and order an assay.

Storage

The miScript Microfluidics PreAMP Kit is shipped on dry ice or cold packs. The kit, including all reagents and buffers, should be stored immediately upon receipt at –20°C in a constant-temperature freezer. If stored under these conditions, the miScript Microfluidics PreAMP Kit is stable for 6 months after receipt.

The miScript Microfluidics PCR Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at –20°C in a constant-temperature freezer. If stored under these conditions, the miScript Microfluidics PCR Kit is stable for 6 months after receipt.

miScript miRNA PCR Arrays are shipped at ambient temperature, on ice, or on dry ice, depending on the destination and the accompanying products. Upon receipt, store at –20°C. If stored under these conditions, miScript miRNA PCR Arrays are stable for 6 months after receipt.

miScript PreAMP Primer Mixes are shipped frozen or at ambient temperature. They should be stored immediately upon receipt in appropriate aliquots at –20°C in a constant-temperature freezer. Avoid repeated freeze–thaw cycles. If stored under these conditions, miScript PreAMP Primer Mixes can be kept for at least 6 months from the date of receipt without any reduction in performance.

miScript Primer Assays are shipped lyophilized at room temperature. Store them at –20°C, either lyophilized or reconstituted (see "Important Notes," page 14). Avoid repeated freeze-thaw cycles. When stored under these conditions and handled correctly, miScript Primer Assays can be kept for at least 18 months from date of receipt without reduction in performance.

miScript Primer Assay 96 Plates are shipped lyophilized at room temperature. Store them at -20°C, either lyophilized or reconstituted (see "Important Notes," page 14). Avoid repeated freeze–thaw cycles. When stored under these conditions and handled correctly, miScript Primer Assay 96 Plates can be kept for at least 18 months from date of receipt without reduction in performance.

Intended Use

The miScript Microfluidics PreAMP Kit, miScript Microfluidics PCR Kit, miScript II RT Kit, miScript PreAMP Primer Mixes, miScript miRNA PCR Arrays, miScript Primer Assays and miScript Primer Assay 96 Plates are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/us/support/qa-qc-safety-data/safety-data-sheets/** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of miScript Microfluidics PreAMP Kit, miScript Microfluidics PCR Kit, miScript II RT Kit, miScript PreAMP Primer Mixes, miScript miRNA PCR Arrays, miScript Primer Assays and miScript Primer Assay 96 Plates is tested against predetermined specifications to ensure consistent product quality.

Introduction

The miScript PCR System allows sensitive and specific detection and quantification of microRNA (miRNA). In contrast to other systems, the miScript PCR System enables detection of an entire species' miRNome from a single cDNA preparation. The miScript PCR System uses total RNA that contains miRNA as the starting material for cDNA synthesis, and separate enrichment of small RNA is not needed or recommended. For more information on miRNA purification, visit **www.qiagen.com/miRNA**.

The miScript Microfluidics PreAMP Kit, miScript Microfluidics PCR Kit, and miScript miRNA PCR Arrays or miScript Primer Assays allow researchers to perform miRNA profiling experiments using the Fluidigm BioMark System together with a 96.96 or 48.48 Dynamic Array IFC. This real-time PCR system enables high-throughput experiments of up to 9216 (96 x 96) or 2304 (48 x 48) reactions on a single chip. The small reaction volume requires careful optimization of reagents and conditions, and adjustments must be made to both input RNA and primer concentrations compared to real-time PCR systems where reactions are performed in 96-well and 384-well plates.

The miScript PCR System for microfluidics represents a breakthrough technology enabling accurate and comprehensive miRNA expression analysis on the Fluidigm BioMark System with as little as 10 ng total RNA. The Fluidigm BioMark System requires a highly multiplex,

PCR-based preamplification. This is routinely performed using up to 400 miRNA-specific cDNA targets in one reaction. Typically, preamplification results in a 1000–4000-fold amplification of each target in the reaction. Optimized and verified miScript PreAMP Primer Mixes are available for every miScript miRNA PCR Array. Preamplification dramatically reduces the sample requirements for analysis. A single 1 ng cDNA synthesis reaction can be used as template for up to 10 preamplification reactions. miScript miRNA PCR Arrays compatible with the Fluidigm BioMark System enable high-throughput assessment of miRNomes and disease- and pathway-focused panels. miScript Primer Assays afford the ultimate flexibility when conducting high-throughput miRNA expression profiling experiments.

Principle and Procedure

miRNA profiling on the Fluidigm BioMark System requires the following 3 steps:

- Reverse transcription using the miScript II RT Kit (see protocol, page 15).
- Preamplification using the miScript Microfluidics PreAMP Kit and miScript PreAMP Primer Mix. This includes a vital side reaction reducer step that minimizes any potential nonspecific reactions in real-time PCR (see protocol, page 18).
- Real-time PCR using either a miScript miRNA PCR Array (see protocol, page 22) or individual miScript Primer Assays (see protocol, page 26) and the miScript Microfluidics PCR Kit.

Reverse transcription

Two buffers, 5x miScript HiSpec Buffer and 5x miScript HiFlex Buffer, are provided in the miScript II RT Kit. miScript HiSpec Buffer facilitates the selective conversion of mature miRNAs into cDNA. miScript HiSpec Buffer is the only buffer that should be used to prepare cDNA for subsequent preamplification with the miScript Microfluidics PreAMP Kit. **Do not use miScript HiFlex Buffer prior to preamplification with the miScript Microfluidics PreAMP Kit**.

For details of how the miScript II RT Kit works, consult the *miScript miRNA PCR Array Handbook*.

Preamplification

Preamplification using the miScript Microfluidics PreAMP Kit and an appropriate miScript PreAMP Primer Mix enables unbiased amplification of the miRNA targets of interest, ensuring that there is sufficient target for quantification in subsequent real-time PCR using the Fluidigm BioMark System. For each miScript miRNA PCR Array, there is a dedicated, verified miScript PreAMP Primer Mix that includes primers to selectively preamplify the miRNAs targeted by the array.

When real-time PCR will be performed with miScript Primer Assays, preamplification must be performed with a miScript PreAMP Primer Mix prepared by pooling the individual miScript Primer Assays (see Appendix A, page 36).

miRNA profiling by real-time PCR

Preamplified cDNA serves as the template for real-time PCR analysis using mature miRNA-specific forward primers (either in miScript miRNA PCR Arrays or as individual miScript Primer Assays) and the miScript Microfluidics PCR Kit, which contains the miScript Microfluidics Universal Primer (reverse primer), and Microfluidics qPCR Master Mix (which is specifically optimized for the Fluidigm BioMark System and contains EvaGreen® dye for detection and ROX™ passive reference dye).

miScript miRNA PCR Arrays (Format M) are specifically designed for the 96.96 Dynamic Array IFCs (Figure 1). This leading miRNA expression profiling technology contains miRNA-specific forward primers, pre-dried at a concentration suitable for use with the Fluidigm BioMark System.

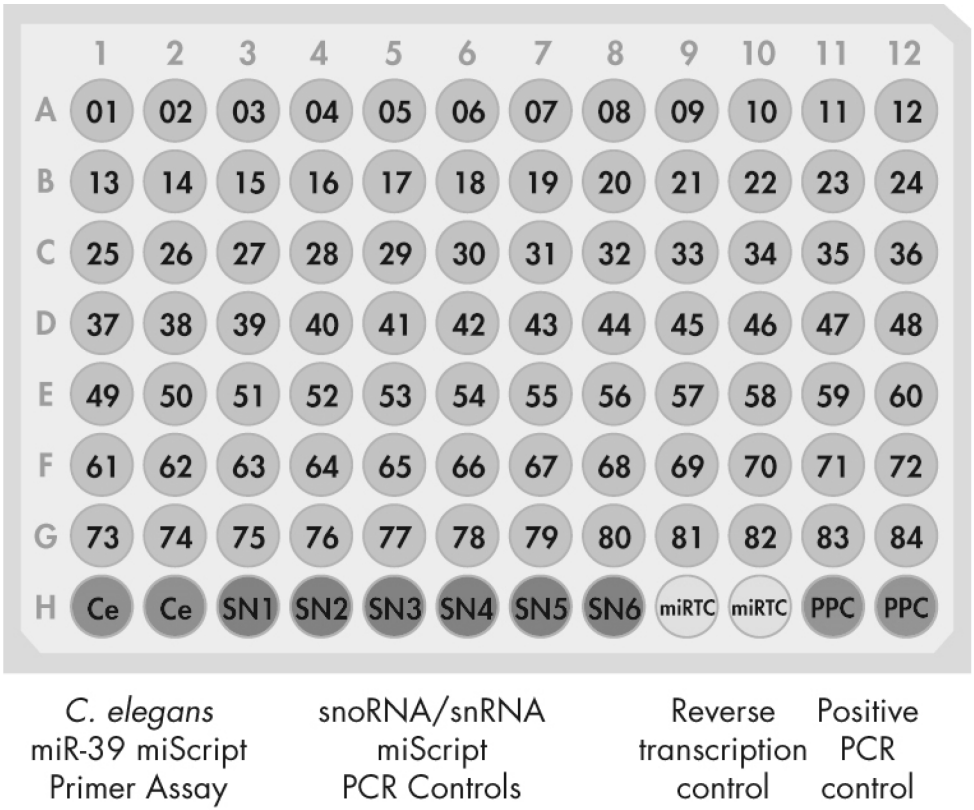

Figure 1. Pathway-focused or miRNome miScript miRNA PCR Array layout for plate format M.
Wells A1 to G12 (**1–84**) each contain a miScript Primer Assay for a pathway/disease/functionally related mature miRNA. Wells H1 and H2 contain replicate *C. elegans* miR-39 miScript Primer Assays that can be used as an alternative normalizer for array data (**Ce**). Wells H3 to H8 each contain an assay for a different snoRNA/snRNA that can be used as a normalization control for the array data (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells H9 and H10 contain replicate miRTC miScript Primer Assays (**miRTC**). Wells H11 and H12 contain replicate positive PCR controls (**PPC**).

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For reverse transcription using the miScript II RT Kit and preamplification using the miScript Microfluidics PreAMP Kit

- Thin-walled, nuclease-free PCR tubes (for 20 µl and 25 µl reactions)
- Ice
- Thermal cycler
- Microcentrifuge

For quantitative, real-time PCR using miScript miRNA PCR Arrays or miScript Primer Assays/miScript Primer Assay 96 Plates

- Fluid BioMark System
- Dynamic Array IFC for Gene Expression (96.96 Dynamic Array IFC [Fluidigm cat. no. BMK-M-96.96] or 48.48 Dynamic Array IFC [Fluidigm cat. no. BMK-M-48.48])
- Control Line Fluid Kit (96.96 [Fluidigm cat. no. 89000021] or 48.48 [Fluidigm cat. no. 89000020])
- 2x Assay Loading Reagent (Fluidigm cat. no. PN 85000736)
- 20x DNA Binding Dye Sample Loading Reagent (Fluidigm cat. no. PN 100-3738)
- 96 x 0.2 ml Plate (BIOplastics cat. no. AB17500)
- EU Optical Wide Area 8-Cap Strip (BIOplastics cat. no. B57801B)
- Multichannel pipettor
- Nuclease-free pipet tips and tubes

Important Notes

Reconstitution of miScript Primer Assay 96 Plates

Two separate dilutions of lyophilized miScript Primer Assay 96 Plates are needed. 100 µM concentration should be used for preparation of miScript PreAMP Primer Mixes (see Appendix A, page 36), and 40 µM concentration should be used for real-time PCR miRNA profiling (see protocol, page 26).

Briefly centrifuge the plate at 1000 rpm to collect the contents at the bottom of each well. Make sure that the centrifugal force is not too high. Using higher than the recommend rpm may cause the plates to break. For plates at the 100-reaction scale, add 27.5 µl RNase-free water to each well (this provides sufficient assay for 16 preamplification reactions and real-time PCRs for the Fluidigm BioMark System). For plates at the 20-reaction scale, add 5.5 µl RNase-free water (this provides sufficient assay for 3 preamplification reactions and real-time PCRs for the Fluidigm BioMark System). Securely and thoroughly seal the plate, mix by vortexing, and briefly centrifuge the plate to collect the contents at the bottom of each well. This results in a concentration of 100 µM. Take an aliquot of miScript Primer Assay 100 µM from each well and further dilute to 40 µM for use in real-time PCR profiling.

Reconstitution of miScript Primer Assays

Two separate dilutions of lyophilized miScript Primer Assay are needed. 100 µM concentration should be used for preparation of miScript PreAMP Primer Mixes (see Appendix A, page 36), and 40 µM concentration should be used for real-time PCR miRNA profiling (see protocol, page 26).

Reconstitute lyophilized miScript Primer Assays with 27.5 µl RNase-free water. This results in a concentration of 100 µM, which should be used for preamplification. Take an aliquot of miScript Primer Assay 100 µM and further dilute to 40 µM for use in real-time PCR profiling.

Protocol: Reverse Transcription Using the miScript II RT Kit

This protocol for reverse transcription using the miScript II RT Kit must be performed prior to preamplification with the miScript Microfluidics PreAMP Kit and subsequent real-time PCR miRNA detection.

Important points before starting

- The miScript II RT Kit includes two 5x buffers: 5x miScript HiSpec Buffer and 5x miScript HiFlex Buffer. Only 5x miScript HiSpec Buffer should be used for reverse transcription prior to preamplification using the miScript Microfluidics PreAMP Kit. **Do not use 5x miScript HiFlex Buffer with the miScript Microfluidics PreAMP Kit**.
- Total RNA containing miRNA should be used as starting material for reverse-transcription reactions. For RNA purification recommendations, visit **www.qiagen.com/miRNA**. This protocol is for use with 10 ng–1 µg RNA in a 10-µl reaction. Recommended RNA amounts should be used to ensure optimal performance. If the RNA sample concentration is not known, we recommend using 5 µl RNA prep in a 10 µl reaction.
- **IMPORTANT**: In this protocol, reverse transcription is performed in a 10 µl reaction volume to maximize the RNA concentration in the reaction. It is essential to perform a 10 µl reverse-transcription reaction prior to preamplification. If a reverse-transcription reaction has already been performed using a 20 µl reaction volume, as described in the miScript PCR System Handbook or the miScript miRNA PCR Array Handbook, and it is not practical to repeat using a 10 µl reaction volume, then this existing reverse-transcription reaction can still be used for preamplification. Dilute the 20 µl reverse-transcription reaction in 80 µl RNase-free water and continue with the protocol on page 18. Do not to change this 1:5 dilution factor.
- Set up all reactions on ice to minimize the risk of RNA degradation.
- Do not vortex template RNA or any of the components of the miScript II RT Kit.

Procedure

1. **Thaw template RNA on ice. Thaw 10x miScript Nucleics Mix, RNase-free water, and 5x miScript HiSpec 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 1. Aliquot 10 µl into each well of a 96-well plate (for 96.96 Dynamic Array IFCs), or into half of the wells of a 96-well plate (for 48.48 Dynamic Array IFCs).**

   The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA.

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

   Note: Master mixes for 48 and 96 samples have volumes 10% greater than that required for the total number of reactions to be performed.

Table 1. Reverse-transcription reaction components

Component	Volume 1 sample	Volume 48 samples	Volume 96 samples
5x miScript HiSpec Buffer	2 µl	106 µl	212 µl
10x miScript Nucleics Mix	1 µl	53 µl	106 µl
RNase-free water (added in step 4)	Variable*	Variable*	Variable*
miScript Reverse Transcriptase Mix	1 µl	53 µl	106 µl
Template RNA (added in step 3)	Variable*†‡	Variable*†‡	Variable*†‡
Total volume	**10 µl**	**530 µl**	**1060 µl**

* If the same volume of template RNA will be added to each sample, a fixed volume of RNase-free water can be included in the reverse transcription master mix instead of adding it in step 4.

† 10 ng – 1 µg total RNA should be used in the reaction to ensure optimal performance.

‡ If RNA was prepared from 100–200 µl serum or plasma using the miRNeasy Serum/Plasma Kit, add 1.5 µl of the RNA prep to the reverse-transcription reaction.

3. Add template RNA to each well containing reverse-transcription master mix. Gently mix, briefly centrifuge and then store on ice.
4. Add RNase-free water to each well containing reverse-transcription master mix and template RNA. Gently mix, briefly centrifuge and then store on ice.
5. Incubate for 60 minutes at 37°C.
6. Incubate for 5 minutes at 95°C to inactivate miScript Reverse Transcriptase Mix, and place on ice.
7. If you wish to proceed with preamplification immediately, dilute the 10 µl cDNA in 40 µl RNase-free water.
8. If you wish to store the reverse-transcription reactions prior to preamplification, transfer the undiluted cDNA to a –20°C freezer, or dispense the diluted cDNA into convenient aliquots and transfer them to a –20°C freezer.

Protocol: Preamplification of cDNA Target Templates

This protocol is for preamplification of cDNA generated using the miScript II RT Kit and miScript HiSpec Buffer. The protocol uses the miScript Microfluidics PreAMP Kit and miScript PreAMP Primer Mix. It should be performed prior to miRNA profiling on the Fluidigm BioMark System.

Important points before starting

- If profiling will be carried out with a miScript miRNA PCR Array, Format M, the corresponding miScript PreAMP Primer Mix is used for preamplification in this protocol. If profiling will be carried out with a miScript Primer Assay 96 Plate or miScript Primer Assays, the miScript PreAMP Primer Mix for preamplification should be prepared as described in Appendix A (page 36).
- **IMPORTANT**: Only cDNA prepared using the miScript II RT Kit with miScript HiSpec Buffer should be used as starting material for this protocol.
- **IMPORTANT**: Use exactly 5 µl diluted cDNA from step 7, page 17 for preamplification as indicated in the protocol. **Using any more or less than 5 µl will adversely affect the performance of the preamplification reaction**. If a reverse-transcription reaction has already been performed using a 20 µl reaction volume, as described in the *miScript PCR System Handbook* or the *miScript miRNA PCR Array Handbook*, 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.

Procedure

1. Thaw 5x miScript PreAMP Buffer, miScript PreAMP Primer Mix, RNase-free water and miScript Microfluidics Universal Primer 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.

   Note: HotStarTaq DNA Polymerase should be removed from the –20°C freezer just before preparation of the master mix, gently mixed, and placed on ice. It should be returned to the freezer immediately after use.

   Note: Master mixes for 48 and 96 samples have volumes 10% greater than that required for the total number of reactions to be performed to allow for small variations in pipetting.

2. **Prepare the preamplification master mix at room temperature (15–25°C) according to Table 2**.

Table 2. Preamplification reaction components

Component	Volume 1 sample	Volume 48 samples	Volume 96 samples
5x miScript PreAMP Buffer	5 µl	265 µl	530 µl
HotStarTaq DNA Polymerase	2 µl	106 µl	212 µl
miScript PreAMP Primer Mix*†	5 µl	265 µl	530 µl
RNase-free water	7 µl	371 µl	742 µl
miScript Microfluidics Universal Primer	1 µl	53 µl	106 µl
Total volume	**20 µl**	**1060 µl**	**2120 µl**

* For some miRNome miScript miRNA PCR Arrays, more than 1 tube of corresponding miScript PreAMP miRNome Primer Mix is provided. Set up separate preamplification reactions for each tube and pool preamplified cDNA prior to use in real-time PCR (see Figure 2**)**.

† When real-time PCR will be performed with individual miScript Primer Assays, a miScript PreAMP Primer Mix prepared using the miScript Primer Assays of interest must be used for preamplification. For details, see Appendix A, page 36.

3. **Mix well, briefly centrifuge, and aliquot 20 µl into each well of a 96-well plate (for 96.96 Dynamic Array IFCs), or into half of the wells of a 96-well plate (for 48.48 Dynamic Array IFCs)**.
4. **Add 5 µl diluted template cDNA to each well containing preamplification master mix. Gently mix, briefly centrifuge and then store on ice**.
5. **Program the thermal cycler according to either Table 3 (for a 96-plex preamplification reaction) or Table 4 (for a 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 200 assays).

   A 384-plex preamplification reaction amplifies 384 cDNA targets and is used with a miScript PreAMP miRNome Primer Mix, or miScript PreAMP Custom Primer Mix (201-384 assays).

Table 3. Cycling conditions for 96-plex preamplification

Step	Time	Temperature
PCR 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 4. Cycling conditions for 384-plex preamplification

Step	Time	Temperature
PCR 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/Extension	3 min	60°C
Cycle Number	10 cycles	

6. **Place the preamplification reaction in the thermal cycler and start the run.**
7. **After the run has finished, add 2 µl Side Reaction Reducer to each preamplified reaction, mix well and centrifuge briefly.**
8. **Incubate at 37°C for 15 minutes, then heat inactivate at 95°C for 5 minutes.**

   It is essential to perform these incubations as described.
9. **Add 98 µl RNase free water. This creates a 5-fold dilution**.

   Note: The optimal dilution can be determined empirically.

   Note: For some miRNome miScript miRNA PCR Arrays, more than 1 tube of corresponding miScript PreAMP miRNome Primer Mix is provided. Pool preamplified cDNA and dilute the pool 5-fold prior to use in real-time PCR. A diagram of this approach is shown in Appendix B, page 37.
10. **Place the diluted reaction at 4°C until ready to continue with real-time PCR, or place at –20°C for long-term storage**.

Protocol: Real-Time PCR miRNA Profiling Using miScript miRNA PCR Arrays

This protocol is for real-time PCR miRNA profiling using the Fluidigm BioMark System. This protocol uses the miScript Microfluidics PCR Kit and the miScript miRNA PCR Array, Format M. Preamplified cDNA generated using the protocol on page 18 is the appropriate starting material for this protocol.

Important points before starting

- **IMPORTANT**: Do not store miScript Microfluidics Universal Primer combined with Fluidigm Assay Loading Reagent. The pre-assay mix (miScript Microfluidics Universal Primer, Assay Loading Reagent and RNase-free water) must be prepared fresh for each experiment.
- **IMPORTANT**: Ensure that contents of the "Assay Plate" or contents of the "Sample Plate" have been added to all assay inlets or sample inlets, respectively, in the Dynamic Array IFC. No inlets can remain empty.

Procedure

1. **Thaw RNase-free water, miScript Microfluidics Universal Primer (40 µM), 2x Assay Loading Reagent, Microfluidics qPCR Master Mix and 20x DNA Binding Dye Sample Loading Reagent at room temperature (15–25°C).**
2. **Prepare the pre-assay mix according to Table 5. Mix thoroughly and briefly centrifuge.**

   Note: Pre-assay mix has a volume 10% greater than that required for the total number of reactions to be performed to allow for small variations in pipetting.

Table 5. Pre-assay mix components

Component	Volume for 96 assays*
miScript Microfluidics Universal Primer (40 µM)	165 µl
2x Assay Loading Reagent (Fluidigm, cat. no. PN 85000736, yellow cap)	330 µl
RNase-free water	165 µl
Total volume	**660 µl**

* To use a 48.48 Dynamic Array IFC with a miScript miRNA PCR Array, adjust the volumes accordingly.

3. **Remove the miScript miRNA PCR Array from its sealed bag.**
4. **Add 6 µl pre-assay mix to each well of the miScript miRNA PCR Array.**

   Note: A multichannel pipettor can be used to add the pre-assay mix to the array.
5. **Carefully, tightly seal the miScript miRNA PCR Array with Optical Thin-Wall 8-Cap Strips and label the array "Assay Plate."**
6. **Vortex the "Assay Plate" thoroughly with 3 pulses of 10 s at full strength. Centrifuge the plate for 2 minutes at 1000 g at room temperature (15–25°C). Set the "Assay Plate" aside until step 15.**
7. **Prepare the pre-sample mix according to Table 6. Mix thoroughly by pipetting up and down. Briefly centrifuge.**

   IMPORTANT: Take care to avoid excessive bubbling when pipetting 20x DNA Binding Dye Sample Loading Reagent.

   Note: Pre-sample mixes for 48 and 96 samples have volumes 10% greater than that required for the total number of reactions to be performed to allow for small variations in pipetting.

Table 6. Pre-sample mix components

Component	Volume 1 sample	Volume 48 samples	Volume 96 samples
Microfluidics qPCR Master Mix	3 µl	165 µl	330 µl
20x DNA Binding Dye Sample Loading Reagent (Fluidigm, PN 100-3738)	0.3 µl	16.5 µl	33 µl
RNase-free water	0.7 µl	38.5 µl	77 µl
Total volume	**4 µl**	**220 µl**	**440 µl**

8. **Using an 8-channel pipet, transfer 4 µl pre-sample mix into each well of a 96-well plate (for 96.96 Dynamic Array IFCs), or into half of the wells of a 96-well plate (for 48.48 Dynamic Array IFCs).**

   Note: We recommend using the 96 x 0.2 ml Plate (BIOplastics, cat. no. AB17500) with EU Optical Wide Area 8-Cap Strips (BIOplastics, cat. no. B57801B) to prepare the plate.

9. **Add 2 µl of each sample (from step 9, page 21) to the appropriate well.**

   The total well volume is 6 µl (4 µl sample mix + 2 µl sample).

10. **Carefully, tightly seal the plate and label the plate "Sample Plate."**

11. Vortex the "Sample Plate" briefly with 1 pulse of 10 s at full strength. Centrifuge for 2 minutes at 1000 x g at room temperature (15–25°C). Set the "Sample Plate" aside in the dark until step 16.

12. Inject control line fluid into each accumulator on the Dynamic Array IFC.

13. Peel the blue protective film from the bottom of the Dynamic Array IFC.

14. Place the Dynamic Array IFC into the IFC controller, then run the "Prime" script to prime the control line fluid into the chip. This takes 10–20 minutes.

15. Dispense 5 µl from the "Assay Plate" from step 6 into the appropriate inlets on the left side of the Dynamic Array IFC.

    IMPORTANT: All inlets on the left side of the Dynamic Array IFC must contain contents from "Assay Plate."

16. Dispense 5 µl from the "Sample Plate" from step 11 into the appropriate inlets on the right side of the Dynamic Array IFC.

    IMPORTANT: All inlets on the right side of the Dynamic Array IFC must contain contents from "Sample Plate."

17. Using the IFC controller, run the "Load" script to load the samples and assays into the reaction chambers of the Dynamic Array IFC. This takes approximately 60–90 minutes.

18. When the "Load" script has finished, remove the loaded Dynamic Array IFC from the IFC controller.

19. Remove any dust or debris from the surface.

20. **Perform cycling as described in Table 7.**

    ROX is used as a passive reference dye.

    Perform dissociation curve analysis as described by Fluidigm.

Table 7. Cycling conditions for real-time PCR

Step	Time	Temperature	Cycle number
Thermal mix* For 96.96 Dynamic Array IFCs only	120 s	50°C	1
	1800 s	70°C	1
	600 s	25°C	1
PCR initial activation step HotStarTaq DNA Polymerase is activated by this heating step.	600 s	95°C	1
3-step cycling:			
	15 s	94°C	
	30 s	55°C	40
Perform fluorescence data collection and dissociation curve analysis.	30 s	70°C	

* If using a 96.96 Dynamic Array IFC, add a thermal mix segment, as recommended by Fluidigm. The thermal mix heats the chip and helps the assay and sample chambers diffuse better on the small chambers on the 96.96 Dynamic Array IFC. A thermal mix is only needed for the 96.96 Dynamic Array IFC and not for the 48.48 Dynamic Array IFC.

Protocol: Real-Time PCR miRNA Profiling Using miScript Primer Assays or miScript Primer Assay 96 Plates

This protocol is for real-time PCR miRNA profiling using the Fluidigm BioMark System. This protocol uses the miScript Microfluidics PCR Kit and miScript Primer Assay 96 Plates or individual miScript Primer Assays. Preamplified cDNA generated using the protocol on page 18 is the appropriate starting material for this protocol.

Important points before starting

- **IMPORTANT**: miScript Primer Assays must be used at a concentration of 40 µM. For details on how to make 40 µM miScript Primer Assay, refer to "Important Notes," page 14.
- **IMPORTANT**: Do not store miScript Microfluidics Universal Primer combined with Fluidigm Assay Loading Reagent. The pre-assay mix (miScript Microfluidics Universal Primer, Assay Loading Reagent, and RNase-free water) must be prepared fresh for each experiment.
- **IMPORTANT**: Ensure that contents of the "Assay Plate" or contents of the "Sample Plate" have been added to all assay inlets or sample inlets, respectively, in the Dynamic Array IFC. No inlets can remain empty.

Procedure

1. **Ensure that the miScript Primer Assays have been reconstituted or diluted to a concentration of 40 µM.**

   For details, see "Important Notes," page 14.

2. **In a 96-well PCR plate, combine an equal volume of miScript Primer Assays (each at 40 µM) with miScript Microfluidics Universal Primer (40 µM).**

   Note: At least 2 µl of each should be combined to provide a miScript Primer Assay/miScript Microfluidics Universal Primer Mix of at least 4 µl.

   Note: We recommend using the 96 x 0.2 ml Plate (BIOplastics, cat. no. AB17500) with EU Optical Wide Area 8-Cap Strips (BIOplastics, cat. no. B57801B) to prepare plates.

3. Carefully, tightly seal the plate, briefly vortex and centrifuge for 2 min at 1000 x g at room temperature (15–25°C).

4. Thaw RNase-free water, miScript Primer Assays mixed with miScript Microfluidics Universal Primer, 2x Assay Loading Reagent, Microfluidics qPCR Master Mix and 20x DNA Binding Dye Sample Loading Reagent at room temperature (15–25°C).

5. **In a new 96-well plate, prepare a pre-assay mix for each target miRNA according to Table 8.**

   Note: Pre-assay mix has a volume 10% greater than that required for the total number of reactions to be performed.

Table 8. Pre-assay mix components

Component	Volume for each well
miScript Primer Assays + miScript Microfluidics Universal Primer from step 2	3 µl
2x Assay Loading Reagent (Fluidigm, cat. no. PN 85000736, yellow cap)	3 µl
Total volume	**6 µl**

6. Carefully, tightly seal the plate and label the plate "Assay Plate."

7. Vortex the "Assay Plate" briefly with 1 pulse of 10 s at full strength. Centrifuge for 2 minutes at 1000 x g at room temperature (15–25°C). Set the "Assay Plate" aside until step 16.

8. Prepare the pre-sample mix according to Table 9. Mix thoroughly by pipetting up and down. Briefly centrifuge.

IMPORTANT: Take care to avoid excessive bubbling when pipetting 20x DNA Binding Dye Sample Loading Reagent.

Note: Pre-sample mixes for 48 and 96 samples have volumes 10% greater than that required for the total number of reactions to be performed to allow for small variations in pipetting.

Table 9. Pre-sample mix components

Component	Volume 1 sample	Volume 48 samples	Volume 96 samples
Microfluidics qPCR Master Mix	3 µl	165 µl	330 µl
20x DNA Binding Dye Sample Loading Reagent (Fluidigm, PN 100-3738)	0.3 µl	16.5 µl	33 µl
RNase-free water	0.7 µl	38.5 µl	77 µl
Total volume of master mix	**4 µl**	**220 µl**	**440 µl**

9. **Using an 8-channel pipet, transfer 4 µl pre-sample mix into each well of a 96-well plate (for 96.96 Dynamic Array IFCs), or into half of the wells of a 96-well plate (for 48.48 Dynamic Array IFCs).**

   Note: We recommend using the 96 x 0.2 ml Plate (BIOplastics, cat. no. AB17500) with EU Optical Wide Area 8-Cap Strips (BIOplastics, cat. no. B57801B) to prepare the plate.

10. **Add 2 µl of each sample (from step 9, page 21) to the appropriate well.**

    The total well volume is 6 µl (4 µl sample mix + 2 µl sample).

11. Carefully, tightly seal the plate and label the plate "Sample Plate."

12. Vortex the "Sample Plate" briefly with 1 pulse of 10 s at full strength. Centrifuge for 2 minutes at 1000 x g at room temperature (15–25°C). Set the "Sample Plate" aside in the dark until step 17.

13. Inject control line fluid into each accumulator on the Dynamic Array IFC.

14. Peel the blue protective film from the bottom of the Dynamic Array IFC.

15. Place the Dynamic Array IFC into the IFC controller, then run the "Prime" script to prime the control line fluid into the chip. This takes 10–20 minutes.
16. **Dispense 5 µl from the "Assay Plate" from step 7 into the appropriate inlets on the left side of the Dynamic Array IFC.**

    IMPORTANT: All inlets on the left side of the Dynamic Array IFC must contain contents from "Assay Plate."
17. **Dispense 5 µl from the "Sample Plate" from step 12 into the appropriate loading wells on the right side of the Dynamic Array IFC.**

    IMPORTANT: All inlets on the left side of the Dynamic Array IFC must contain contents from "Sample Plate."
18. Using the IFC controller, run the "Load" script to load the samples and assays into the reaction chambers of the Dynamic Array IFC. This takes approximately 60–90 minutes.
19. When the "Load" script has finished, remove the loaded Dynamic Array IFC from the IFC controller.
20. Remove any dust or debris from the surface.
21. **Perform cycling as described in Table 10.**

    ROX is used as a passive reference dye.

    Perform dissociation curve analysis as described by Fluidigm.

Table 10. Cycling conditions for real-time PCR

Step	Time	Temperature	Cycle number
Thermal mix*	120 s	50°C	1
For 96.96 Dynamic Array IFCs only			
	1800 s	70°C	1
	600 s	25°C	1
PCR initial activation step HotStarTaq DNA Polymerase is activated by this heating step.	600 s	95°C	1
3-step cycling:			
	15 s	94°C	
	30 s	55°C	40
Perform fluorescence data collection and dissociation curve analysis.	30 s	70°C	

* If using a 96.96 Dynamic Array IFC, add a thermal mix segment. The thermal mix heats the chip and helps the assay and sample chambers diffuse better on the small chambers on the 96.96 Dynamic Array IFC. A thermal mix is only needed for the 96.96 Dynamic Array IFC and not for the 48.48 Dynamic Array IFC.

Protocol: Data Analysis for miScript miRNA PCR Arrays

Procedure

Steps performed by the user

1. In the Fluidigm real-time PCR analysis software, ensure the "Quality Threshold" is set at 0.65.
2. Select "Linear (Derivative)" for Baseline Correction.
3. Select "User (Global)" for C_T Threshold Method.
4. Define the Threshold (EvaGreen).

   We recommend using a Threshold 0.0077 as a starting point.

   Note: Ensure that threshold settings are the same across all PCR runs in the same analysis to allow comparison of results.
5. Export the C_T values.
6. Data analysis can then be conducted at QIAGEN's GeneGlobe Data Analysis Center using a software-based tool.

   Note: The GeneGlobe Data Analysis Center is a web resource for the analysis of real-time PCR or NGS data (**www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/?akamai-feo=off**). To access the center, new users can register online. Once on the site, the data analysis software will be found under "Analysis." The miScript miRNA PCR Data Analysis spreadsheets can be found under "Product Resources/Performance Data" at **www.qiagen.com/us/shop/pcr/primer-sets/miscript-mirna-pcr-arrays/#resources**.

   Note: If using a 384-well format, download the PCR Array 4x96 384 Well Conversion spreadsheet to dissect a 384-well dataset into the correct 4 sets of 96 genes for each of the 4 samples. For software-based data analysis, this file can be found at the GeneGlobe

Data Analysis Center. For spreadsheet-based data analysis, this file can be found under "Product Resources/Performance Data" at **www.qiagen.com/us/shop/pcr/primer-sets/miscript-mirna-pcr-arrays/#resources**.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQlist.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

		Comments and suggestions
Evidence of poor reverse transcription efficiency (value of AVG C_T^{miRTC} – AVG C_T^{PPC} higher than expected)		
a)	Poor quality RNA or reverse transcription inhibitors present in RNA	Check the A_{260}:A_{280} and A_{260}:A_{230} ratios of the RNA samples. Be sure to perform the dilutions for spectrophotometry in RNase-free 10 mM Tris·Cl, pH 7.5. If necessary, repurify RNA with a spin-column based clean up method, such as the miRNeasy Mini Kit (cat. no. 217004).
b)	Volume of reverse-transcription reaction added to the preamplification reaction was too high or too low	It is important to add the exact recommended volume of reverse-transcription reaction to the preamplification reaction to guarantee optimal specificity and efficiency. Repeat the preamplification reaction using the correct volume of reverse-transcription reaction (5 µl of a 10 µl reverse-transcription reaction that has been diluted to 50 µl; see "Important points before starting," page 18).
c)	Incorrect buffer used during reverse transcription	Ensure that 5x miScript HiSpec Buffer is used for reverse transcription prior to preamplification using the miScript Microfluidics PreAMP Kit.
d)	Inefficient preamplification due to incorrect cycling	Ensure that cycling conditions in Table 3 are used for 96-plex preamplification reactions, and cycling conditions in Table 4 are used for 384-plex preamplification reactions.
Evidence of poor overall PCR amplification efficiency (AVG C_T^{PPC} varies by more than 2 across arrays and/or is greater than 15)		
a)	Variation in instrument sensitivity	Different instruments have different levels of sensitivity. If an average C_T^{PPC} value of 13 ± 2 is difficult to obtain for the instrument used, the observed average C_T^{PPC} value should be acceptable as long as it does not vary by more than 2 cycles between arrays being compared.
b)	HotStarTaq DNA Polymerase not activated with a hot start	Be sure that the initial heat activation step at 95°C took place for 15 minutes, and that all other cycle parameters were performed according to the protocol.

		Comments and suggestions
c)	Poor quality RNA that may contain PCR inhibitors	Check the A_{260}:A_{280} and A_{260}:A_{230} ratios of the RNA samples. Be sure to perform the dilutions for spectrophotometry in RNase-free 10 mM Tris·Cl, pH 7.5. If necessary, repurify RNA with a spin-column based clean up method, such as the miRNeasy Mini Kit (cat. no. 217004).
Fluidigm BioMark run is unsuccessful or displays the error message: "Corners in the chip weren't detected. Would you like to manually find them?"		
a)	Bubbles introduced during loading of the Dynamic Array IFC inlets	Avoid bubbles during loading of the Dynamic Array IFC inlets. When pipetting, do not continue to press the plunger after the first stop has been reached.
b)	Assay and sample loading were reversed (i.e., contents of the "Assay Plate" were loaded on the right side of the Dynamic Array IFC and contents of the "Sample Plate" were loaded on the left side of the Dynamic Array IFC)	Ensure that contents of the "Assay Plate" are loaded on the left side of the Dynamic Array IFC and contents of the "Sample Plate" are loaded on the right side of the Dynamic Array IFC.
c)	Assay and/or sample inlets are empty	Ensure that contents of the "Assay Plate" or contents of the "Sample Plate" have been added to all assay inlets or sample inlets, respectively, in the Dynamic Array IFC.
d)	Thermal mix step not included during 96.96 Dynamic Array IFC experiment	Ensure that thermal mix step has been included in real-time PCR cycling for the 96.96 Dynamic Array IFC.
High background signal observed for all samples/assays in real-time PCR		
	Side reaction reducer step at the end of preamplification was not performed.	Ensure that the side reaction reduction step (step 7, page 21) has been performed during preamplification.
No product, or product detected late in real-time PCR (indicative of problems occurring during reverse transcription)		
a)	Pipetting error or missing reagent when setting up reverse-transcription reaction	Check the pipets used for experimental setup. Mix all reagents well after thawing and repeat the reverse-transcription reaction.

		Comments and suggestions
b)	Poor quality or incorrect amount of template RNA for reverse-transcription reaction	Check the concentration, integrity, and purity of the template RNA before starting the protocol. Mix well after thawing the template RNA. Even minute amounts of RNases can affect synthesis of cDNA and sensitivity in RT-PCR, particularly with small amounts of RNA.
c)	RNA concentration too high or too low	The miScript II RT Kit in combination with the miScript PreAMP PCR Kit are intended for use with 10 ng to 1 µg RNA.
d)	Incubation temperature too high	Reverse transcription should be carried out at 37°C. Higher temperatures may reduce the length of cDNA products or the activity of miScript Reverse Transcriptase Mix. Check the temperature of your heating block or water bath.

No product, or product detected late in real-time PCR, or only primer-dimers detected (indicative of problems occurring during real-time PCR)

a)	Incorrect storage of Microfluidics qPCR Master Mix	Microfluidics qPCR Master Mix should be stored immediately upon receipt at –20°C in a constant-temperature freezer.
b)	Volume of reverse-transcription reaction added to the preamplification reaction was too high or too low	It is important to add the exact recommended volume of reverse-transcription reaction to the preamplification reaction to guarantee optimal specificity and efficiency. Repeat the preamplification reaction using the correct volume of reverse-transcription reaction (5 µl of a 10 µl reverse-transcription reaction that has been diluted to 50 µl; see "Important points before starting," page 18).
c)	Pipetting error or missing reagent when setting up PCR	Check the concentrations and storage conditions of reagents, including primers and cDNA.
d)	HotStarTaq DNA Polymerase not activated with a hot start	Ensure that the cycling program includes the hot start activation step for HotStarTaq DNA polymerase; check the protocol for details.
e)	No detection activated	Check that fluorescence detection was activated in the cycling program.
f)	Wrong detection step	Ensure that fluorescence detection takes place during the extension step of the PCR cycling program.

Appendix A: Preparation of miScript PreAMP Primer Mixes from miScript Primer Assays

miScript PreAMP Primer Mixes are available for all miScript miRNA PCR Arrays. Alternatively, preamplification can be performed using the miScript PreAMP PCR Kit together with a mix of miScript Primer Assays prepared by the user, as described in this protocol.

Procedure

1. **Reconstitute lyophilized miScript Primer Assays to 100 µM as described in "Important Notes," page 14.**
   If miScript Primer Assay stocks are at a lower concentration, adjust the protocol accordingly.
   Do not reconstitute miScript Primer Assays as described in the *miScript PCR System Handbook*. This would result in a 5 µM concentration, which is not suitable for use with the Fluidigm BioMark System.
2. **Combine and dilute miScript Primer Assay(s) to prepare miScript PreAMP Primer Mixes for up to 384 miRNAs as described in Table 11.**
   IMPORTANT: Do not pool more than 384 miScript Primer Assays in one miScript PreAMP Primer Mix.

Table 11. Preparation of miScript PreAMP Primer Mix for 2 x 96.96 Dynamic Array IFCs

Number of miRNAs	miScript Primer Assay	RNase-free water
1 miRNA	1 assay x 2.2 µl = 2.2 µl	1098 µl
2 miRNA	2 assays x 2.2 µl each = 4.4 µl	1096 µl
96 miRNA	96 assays x 2.2 µl each = 211.2 µl	889 µl
384 miRNA	384 assays x 2.2 µl each = 844.8 µl	255 µl

3. Dilute an aliquot of 100 µM miScript Primer Assay from step A1 to 40 µM for use in real-time PCR (see protocol, page 26).

Appendix B: Preamplification Using miScript PreAMP miRNome Primer Mixes in Multiple Tubes

miScript PreAMP miRNome Primer Mixes may be provided in multiple tubes. In these cases, it is necessary to set up separate preamplification and side reaction reduction reactions and pool once the reactions are completed. For example, if 5 tubes of miScript PreAMP miRNome Primer Mix are provided, set up 5 reactions — one for each tube. After the side reaction reduction step, pool the 5 reactions into one tube.

Example miScript PreAMP miRNome Primer Mix for Human miRNome V18

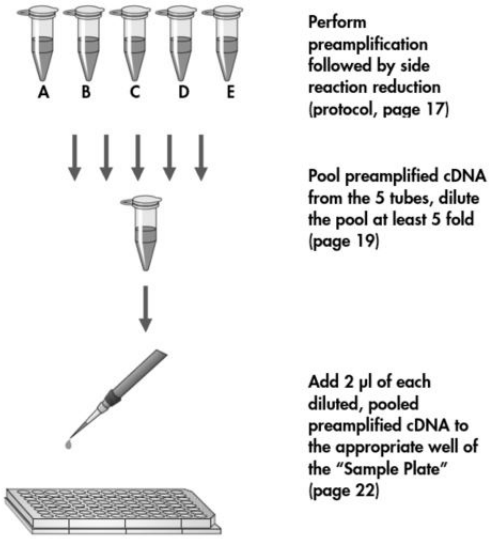

Figure 2. Preamplification using miScript PreAMP miRNome Primer Mixes in multiple tubes. Preamplification reactions are set up for each tube of primer mix, and the preamplified cDNA is then pooled and diluted at least 5-fold prior to being aliquoted into the sample plate.

Ordering Information

Product	Contents	Cat. no.
miScript Microfluidics PreAMP Kit (96)	For 96 reactions: 5x miScript PreAMP Buffer, HotStarTaq DNA Polymerase (2 U/µl), miScript PreAMP Universal Primer (10 µM), SR1 Reagent	331455
miScript Microfluidics PCR Kit (5)	For 5 x 96.96 Dynamic Array IFCs: Microfluidics qPCR Master Mix, miScript Microfluidics Universal Primer (40 µM)	331431
miScript II RT Kit (12)	For 12 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water	218160
miScript II RT Kit (50)	For 50 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water	218161
miScript Primer Assay 96 Plate	Customer-configured miScript Primer Assays in 96-well plates. Content may be chosen by the customer at **www.qiagen.com/GeneGlobe**. Two synthesis scales are available.	218540
miScript PreAMP Pathway Primer Mix	530 µl primer mix for preamplification; for use with a Pathway-Focused miScript miRNA PCR Array	Varies

miScript PreAMP miRNome Primer Mix	530 µl/tube primer mix for preamplification; for use with a miRNome miScript miRNA PCR Array	Varies
miScript PreAMP Custom Primer Mix	1060 µl/tube primer mix for preamplification; for use with a Custom miScript miRNA PCR Array	Varies
miScript Primer Assay (100)	miScript Primer Assay (contains one miRNA-specific primer)	Varies
miScript miRNA PCR Array, Format M	Single-use miRNome or biology-focused panels of miRNA primer assays ready for use with the Fluidigm BioMark System	Varies
Related products		
miRNeasy Micro Kit (50)	For 50 total RNA preps: 50 RNeasy® MinElute® Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol® Lysis Reagent, RNase-Free Reagents and Buffers	217084
miRNeasy Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004
miRNeasy 96 Kit (4)	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
miRNeasy Serum/Plasma Kit (50)	For 50 total RNA preps: 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, Ce_miR-39_1 miScript Primer	217184

	Assay, RNase-free Reagents and Buffers	
miRNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	217504

* Larger kit sizes available; please inquire.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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