# miScript® PreAMP PCR Kit

## For miRNA profiling from limiting RNA samples

The miScript PreAMP PCR Kit enables highly multiplex, PCR-based preamplification to facilitate use of the miScript PCR System for miRNA quantification with starting samples containing extremely low amounts of RNA. The miScript PreAMP PCR Kit, used with miScript PreAMP Primer Mixes, can provide sufficient template for miRNA profiling with multiple pathway- or disease-focused miScript miRNA PCR Arrays or multiple replicates of a miRNome miScript miRNA PCR Array.

**Advantages of the miScript PreAMP PCR Kit:**

- Enabling technology for miRNA profiling from FFPE, serum/plasma, LCM, and body fluid samples
- Profile multiple pathways or an entire miRNome using 10 ng or less total RNA
- Integrated controls to evaluate preamplification efficiency
- Easy procedure integrated into the miScript PCR System workflow

## Breakthrough technology for miRNA profiling

The miScript PreAMP PCR Kit, a component of the miScript PCR System, allows researchers to perform miRNA profiling experiments using very limited amounts of starting RNA (see Janas, M.M. et al. (2012) Reduced Expression of Ribosomal Proteins Relieves MicroRNA-Mediated Repression. Molecular Cell **46**, 171). This is necessary when working with samples that contain low amounts of RNA, such as body fluids, formalin-fixed, paraffin-embedded (FFPE) samples, and low cell number samples, such as laser capture microdissection (LCM) samples, flow-sorted cells, circulating tumor cells, and fine-needle biopsies.

The miScript PreAMP PCR Kit, used with miScript PreAMP Primer Mixes, is a breakthrough technology enabling accurate and comprehensive expression analysis with 10 ng or less total RNA. A single 10 ng cDNA synthesis reaction can be used as template for up to 10 preamplification reactions. This provides sufficient template for miRNA profiling using multiple pathway- or disease-focused miScript miRNA PCR Arrays or multiple screenings of miRNome miScript miRNA PCR Arrays, depending on the miScript PreAMP Primer Mix used. The miScript PreAMP PCR Kit uses highly multiplex, PCR-based preamplification of up to 400 miRNA-specific cDNA targets in one reaction. Typically, preamplification results in a 2000–4000-fold amplification in either a 96-plex or 384-plex reaction.

## Integrated miScript PCR System workflow

miRNA profiling from low-RNA–content samples with the miScript PCR System is based on SYBR® Green real-time PCR. The following 3 steps are performed: reverse transcription using the miScript II RT Kit, preamplification using the miScript PreAMP PCR Kit and miScript PreAMP Primer Mix, ▶

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and real-time PCR using the corresponding miScript miRNA PCR Array and the miScript SYBR Green PCR Kit. Preamplification using the miScript PreAMP PCR Kit and miScript PreAMP Primer Mix is therefore fully integrated into the miScript PCR System miRNA profiling workflow, ensuring robust protocols and reliable, reproducible results.

## Fully controlled experiments

The miScript PreAMP PCR Kit includes 4 miScript Primer Assays for use in preamplification control experiments to test preamplified cDNA (see Table 1; for more information, see the *miScript PreAMP Handbook*). Use of appropriate controls ensures reliable, easy-to-interpret results.

**Table 1. Controls in miScript PreAMP PCR Kit**

| Control | Purpose |
|---|---|
| miR-16 miScript Primer Assay | Determination of the optimal dilution factor for preamplified cDNA if the template starting amount is unknown |
| SNORD95 miScript Primer Assay | Data normalization for tissue and cell samples using the $\Delta\Delta C_T$ method of relative quantification<br>Determination of the optimal dilution factor for preamplified cDNA if the template starting amount is unknown and miR-16 is not present |
| *C. elegans* miR-39 miScript Primer Assay | Measurement of miRNeasy Serum/Plasma Spike-In Control (if added during purification) for determination of recovery from serum and plasma samples |
| miRTC miScript Primer Assay | Assessment of reverse-transcription efficiency |

## Profile miRNA changes from less than 1 µl serum or plasma

The miScript PreAMP PCR Kit offers the opportunity to achieve large amounts of reliable miRNA profiling data from small samples and samples with very low RNA content. Previously unusable samples can now provide valuable information about miRNA expression and function. Using preamplification, less than 0.1 µl serum equivalence RNA was used to robustly profile miRNA expression using the Serum & Plasma miScript miRNA PCR Array (Figure 1).

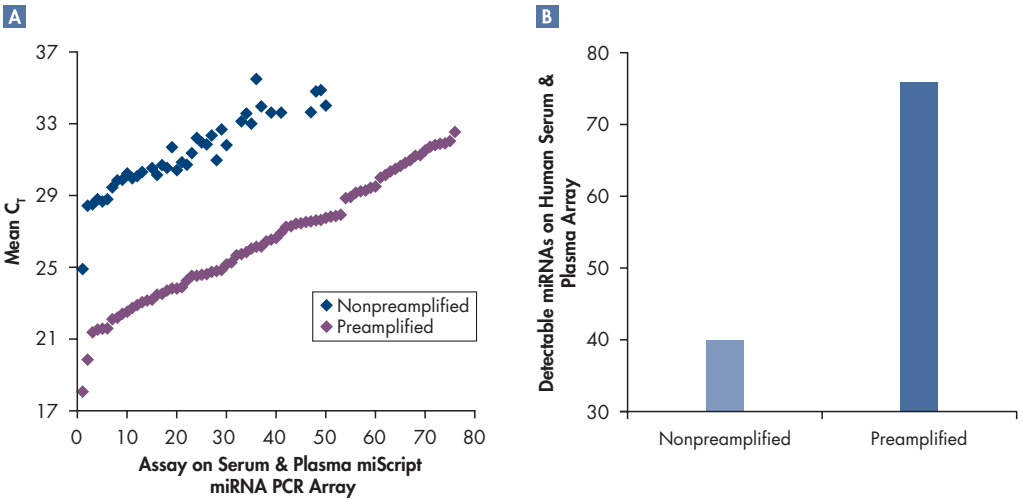


**Figure 1. 100% increase in miRNAs detected from 10-fold less cDNA.** Total RNA was purified from 5 µl human serum using the miRNeasy Serum/Plasma Kit. cDNA was then prepared from 0.7 µl serum equivalence (SE) using the miScript II RT Kit with miScript HiSpec Buffer. cDNA (0.7 µl SE) was used directly for miRNA profiling or one-tenth of the cDNA preparation (0.07 µl SE) was preamplified using the miScript PreAMP PCR Kit with Serum & Plasma miScript PreAMP Pathway Primer Mix prior to profiling. miRNA profiling was performed with the with the Serum & Plasma miScript miRNA PCR Array. Plots of A mean $C_T$ values achieved and B number of miRNAs detected demonstrate highly superior results from 10-fold less starting cDNA due to preamplification.

## High correlation with results from nonpreamplified samples

Reliable, unbiased amplification using the miScript PreAMP PCR Kit provides data that correlates highly with nonpreamplified sample, ensuring high confidence in results. Comparison of miRNA profiling results from tumor and normal lung tissue FFPE samples demonstrated this strong correlation (Figure 2).

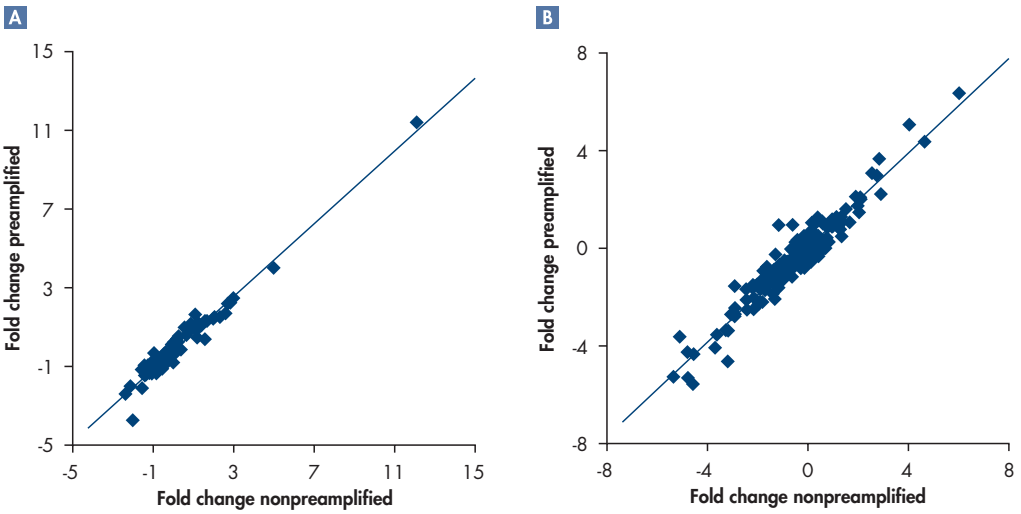


**Figure 2. Preamplification preserves expression patterns using 1000-fold less input cDNA.** Preamplified cDNA and nonpreamplified cDNA from the same prep were used for miRNA profiling. Scatter plots of x-fold expression change calculations ($2^{-\Delta\Delta C_T}$) between normal and tumor sections demonstrate high correlation between nonpreamplified and preamplified samples. A 96-plex miFinder miScript PreAMP Pathway Primer Mix and Array or B 384-plex miScript PreAMP miRNome Primer Mix and Array were used. The miRNeasy FFPE Kit was used to purify RNA from normal and tumor lung tissue 5 µM FFPE sections. cDNA was prepared from 10 ng total RNA using the miScript II RT Kit with miScript HiSpec Buffer and preamplified using the miScript PreAMP PCR Kit.

## Reproducible results and robust procedure

Preamplification using the miScript PreAMP PCR Kit is a robust procedure that ensures consistent, reproducible results. Highly overlapping results were achieved when preamplification and miRNA profiling experiments were performed by different users (Figure 3).

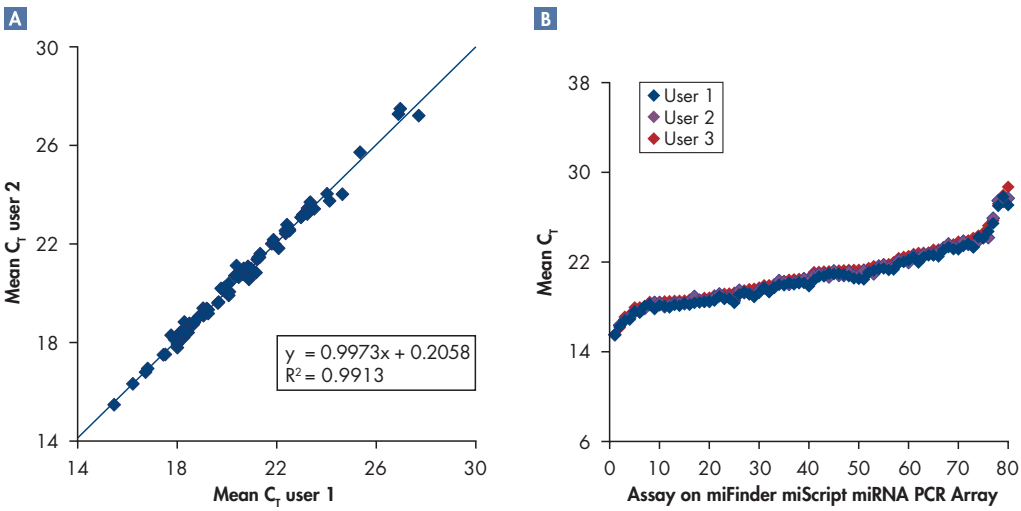


**Figure 3. High technical reproducibility.** cDNA was prepared from a human universal reference RNA sample using the miScript II RT Kit with miScript HiSpec Buffer. Three different users performed preamplification using the miScript PreAMP PCR Kit and miFinder miScript PreAMP Pathway Primer Mix. Preamplified cDNA was used for miRNA profiling in duplicate with the miFinder miScript miRNA PCR Array. High levels of correlation were observed between users when $C_T$ values were A compared on a scatter plot or B plotted for all 3 users.

## Wide range of applications

The miScript PreAMP PCR Kit is used as part of the miScript PCR System for:

- Mature miRNA quantification and profiling from limiting samples
- Biomarker discovery from serum or plasma, other body fluids, or archival FFPE samples

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| miScript PreAMP PCR Kit (12) | Enzyme, reagents, and controls for 12 preamplification reactions | 331451 |
| miScript PreAMP PCR Kit (60) | Enzyme, reagents, and controls for 60 preamplification reactions | 331452 |
| miScript PreAMP Pathway Primer Mix | 60 µl primer mix for 12 preamplification reactions; for use with a Pathway-Focused miScript miRNA PCR Array | Varies |
| miScript PreAMP Pathway HC Primer Mix | 60 µl primer mix for 12 preamplification reactions; for use with a miScript miRNA HC PCR Array | Varies |
| miScript PreAMP miRNome Primer Mix | 60 µl/tube primer mix for 12 preamplification reactions; for use with a miRNome miScript miRNA PCR Array | Varies |
| miScript PreAMP Custom Primer Mix | 100 µl/tube primer mix for 80 preamplification reactions; for use with a Custom miScript miRNA PCR Array | Varies |

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.

**Discover more at www.qiagen.com/miScript-PreAMP!**



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