Product Profile

miScript® Single Cell qPCR Kit

For isolation of miRNA from limited samples as low as a single cell

The miScript Single Cell qPCR Kit enables:

- Universal miRNA quantification with an all-in-one box kit
- miRNA expression profiling from single cells
- Sample to Insight® experiments with integrated analysis through GeneGlobe® Data Analysis Center

Precision tools for miRNA quantification from a single cell

Single cells represent the ultimate in precious samples, as no two cells are the same. miRNA quantification from single cells offers the ultimate ability to identify key expression differences between varying individual cell types. miRNAs have already been identified as key biomarkers in cell-free and exosomal applications, and play prominent roles in virtually all areas of normal and disease biology. With the miScript Single Cell qPCR Kit, you can unlock miRNAs signatures and heterogeneity locked in single cells. Precise single cell quantification of miRNA from a single cell; the possibilities are endless.

The miScript Single Cell qPCR Kit relies on a highly optimized, adapter ligation-based cDNA synthesis and amplification method that virtually eliminates artificial quantification bias. The kit liberates and enables miRNA quantification using a robust, highly optimized method that maximizes sensitivity. A unique approach enables miRNA specific, universal amplification for downstream quantification from an individual miScript miRNA PCR Assay up to an entire miRNome.

Unbiased universal workflow

The high sensitivity, reproducibility and precision of the miScript Single Cell qPCR Kit is achieved by taking advantage of the unique properties of mature miRNAs. Animal mature miRNAs possess a 3′ hydroxyl group and a 5′ phosphate group (unlike most other cellular RNAs). Due to this, adapters can be ligated to both the 3′ end and 5′ end of mature miRNAs enabling universal reverse-transcription and preamplification, while minimizing the background from other RNA ▷

species. The buffers and enzymes used during the ligation, reverse-transcription and preamplification reactions have all been optimized extensively for enzymatic reactions involving low RNA amounts found in single cells.

Robust and reliable miRNA quantification from single cells offer the ultimate ability to identify individual differences between varying individual types.

- Circulating tumor cells
- Cells from varying treatments
- Immune cells

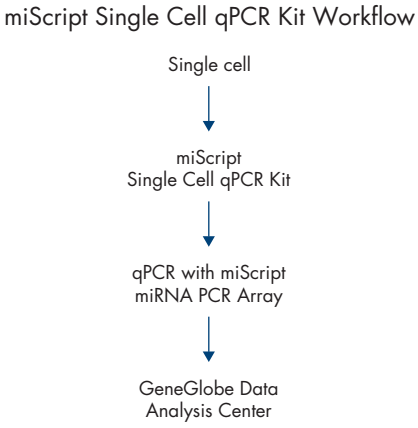

Figure 1. miScript Single Cell qPCR Kit Workflow. A one box solution allows you to take a single cell, lyse and perform unbiased universal miRNA cDNA synthesis and universal amplification. This enables any qPCR application downstream from a single assay to entire miRNome.

miRNA Single Cell Applications

Analyzing single cells can uncover inherent variability amongst individual cells. Circulating tumor cells can vary tremendously from primary tumor. Substantial changes in these cells can yield important differences that are important in transformation and metastasis. Circulating tumor cells and individually treated cell cultures are just two of the many applications the miScript Single Cell Kit was designed for. Cells can vary tremendously between treatments, or between free and tumor bound. With the GeneGlobe Data Analysis Center, the heterogeneity of individual cells can be deciphered using the built-in principle component analysis (PCA) (Figure 2). PCA enables grouping of similar cells based on the heterogeneous miRNA signatures, enabling direct cell-to-cell comparison of miRNA transcripts. The miScript Single Cell Kit enables sensitive detection of low copy number miRNA, enabling access to not only high abundance targets but also rarer transcripts lost with less sensitive technology (Figure 3).

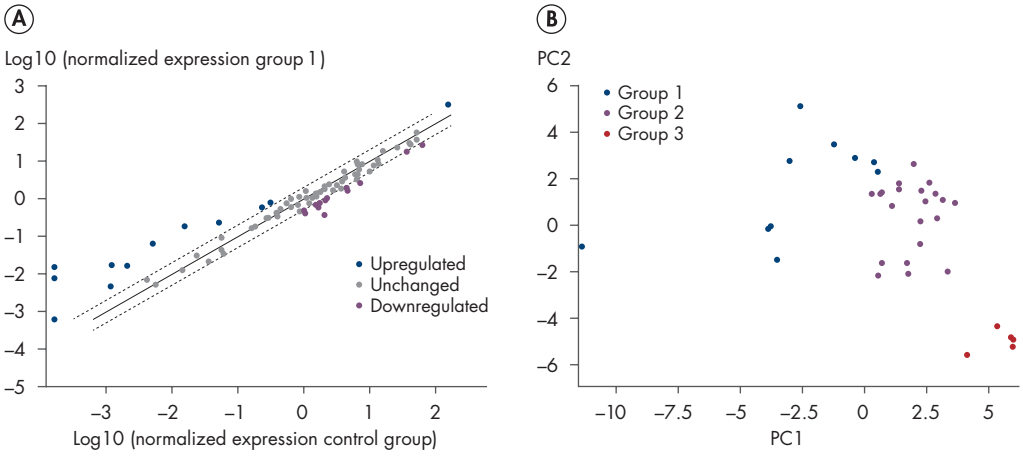

Figure 2. PCA reveals a heterogeneous response amongst individual cells to 5-aza-2′-deoxycytidine treatment. HCT 116 colorectal cancer cells were treated ± 5-aza-2′-deoxycytidine (5-aza-2′-dC) demethylation reagent. Bulk cell pellets (**Panel A**) and individually isolated treated cells (**Panel B**) were processed using the miScript Single Cell qPCR Kit, and the miFinder miScript miRNA PCR Array was used in real-time PCR. For the bulk sample analysis (Panel A), a scatter plot of $2^{-\Delta CT}$ values demonstrates that differential miRNA expression was observed in response to the 5-aza-2′-dC treatment. PCA analysis of individual cells (Panel B) reveals a differential response amongst the treated cells with three distinct populations. Together, the data suggests the consequential "cellular averages" of bulk analyses mask intrinsic transcriptional variability across individual cells.

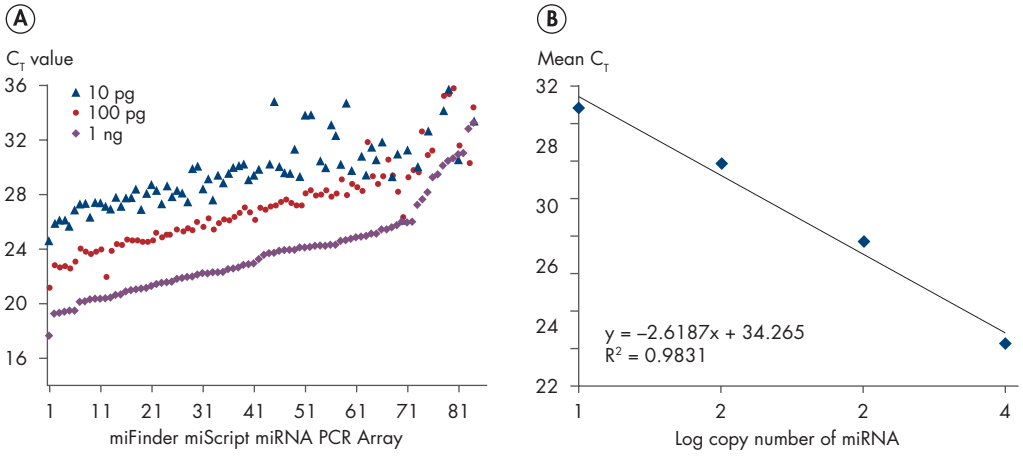

Figure 3. The miScript Single Cell qPCR Kit enables sensitive detection of low abundance miRNAs. A 10-fold dilutions of HeLa S3 purified RNA (1 ng, 100 pg and 10 pg) were processed using the miScript Single Cell qPCR Kit, and the human miFinder miScript miRNA PCR Array was used in real-time PCR. **B** 10-fold dilutions ($1x10^4$ copies to 10 copies) of a pool of 24 synthetic miRNAs were processed using the miScript Single Cell qPCR Kit, and miScript Primer Assays were used in real-time PCR. With increasing 10-fold dilutions of RNA or synthetic miRNAs, the C_T unit decreased accordingly demonstrating the sensitive detection capacity of the miScript Single Cell qPCR Kit.

Ordering Information

Product	Contents	Cat. no.
miScript Single Cell qPCR Kit – Quantify miRNA directly from as low as a single cell		
miScript Single Cell qPCR Kit (24)	Isolate miRNA from as low as a single cell to quantify single miRNA up to the entire miRNome. Available in 24 or 96 cell pack sizes	331053
miScript Single Cell qPCR Kit (96)	Includes: lysis buffer, adapters, preamp mix	331055
QIAGEN miScript miRNA PCR System		
miScript miRNome PCR Array	Profile all available miRNA from human, mouse or rat – V16	331222
miScript miRNA PCR Array	miRNA PCR arrays for pathways and diseases for multiple species	331221
miScript miRNA miRBase Profiler	Profile all the most up to date human miRNome, 2402 Targets Available in 384-well format	331223
GeneGlobe Data Analysis Center	Free data analysis software tailored for single-cell miRNA expression analysis is available at www.qiagen.com/GeneGlobe. The analysis software performs principle component analysis (PCA) and hierarchical clustering, and results are presented in a variety of visual formats	Free with array
Ingenuity® Pathway Analysis	Upload GeneGlobe DAC output directly into Ingenuity Pathway Analysis to interpret complex interactions between cells and pathways–not included	Separate purchase*

* Requires purchase or access to Ingenuity Pathway Analysis

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 the power of a single cell. Visit **www.qiagen.com/miScript-single-cell**.