User's Manual and Instructions

Product: miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200)
miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200)

Catalog Number: KS081200, KS082200

Introduction

MicroRNAs (miRNAs) are endogenous regulators of gene expression that are encoded in the genomes of plants, animals and viruses, then processed into ~19-23 nt, single-stranded molecules that become incorporated into the RNA-induced silencing complex (RISC). RISC mediates down-regulation of gene expression through translational inhibition, transcript cleavage, or both. Designed for use with BioChain's MicroRNA qRT-PCR primer sets, the MicroRNA One-Step qRT-PCR Detection Kit provides a one-step, simple, robust, inexpensive assay for quantitative analysis of miRNA expression from total RNA samples, or small RNAs enriched samples. In this kit, EvaGreen dye, a superior green fluorescence DNA-binding dye is used for real-time detection and quantification of DNA. The short length of miRNAs presents a challenge in PCR design. BioChain's microRNA qRT-PCR primer sets were uniquely designed and optimized for detecting mature miRNA with high specificity and sensitivity at low PCR annealing temperature. The primer set includes a forward PCR primer and a reverse PCR primer. The reverse PCR primer is also served as RT primer for reverse transcription. This kit contains an enzyme mix, a 2x- concentrated qRT-PCR reaction mixture and a miRNA primer set that contain all the reagents (except templates) needed for running qRT-PCR assay. The passive reference dye ROX is included in a separate tube to make this kit adaptable for many real-time QPCR platforms. The kit includes the hsa-miR-24 (Cat# KS081200) or hsa-miR-16 (Cat# KS081200) qRT-PCR primer set for amplification of the widely expressed miR-24 or miR-16 from either the Human Placenta Total RNA supplied with the kit or from user-supplied human, mouse and rat RNA samples (Figure 1 and 2).

BioChain also provide custom service to design specific microRNA qRT-PCR primer sets for using with this one-step qRT-PCR microRNA detection system. For researcher interested in detecting other microRNAs besides miR-24 and miR-16, please contact BioChain Technical Support.

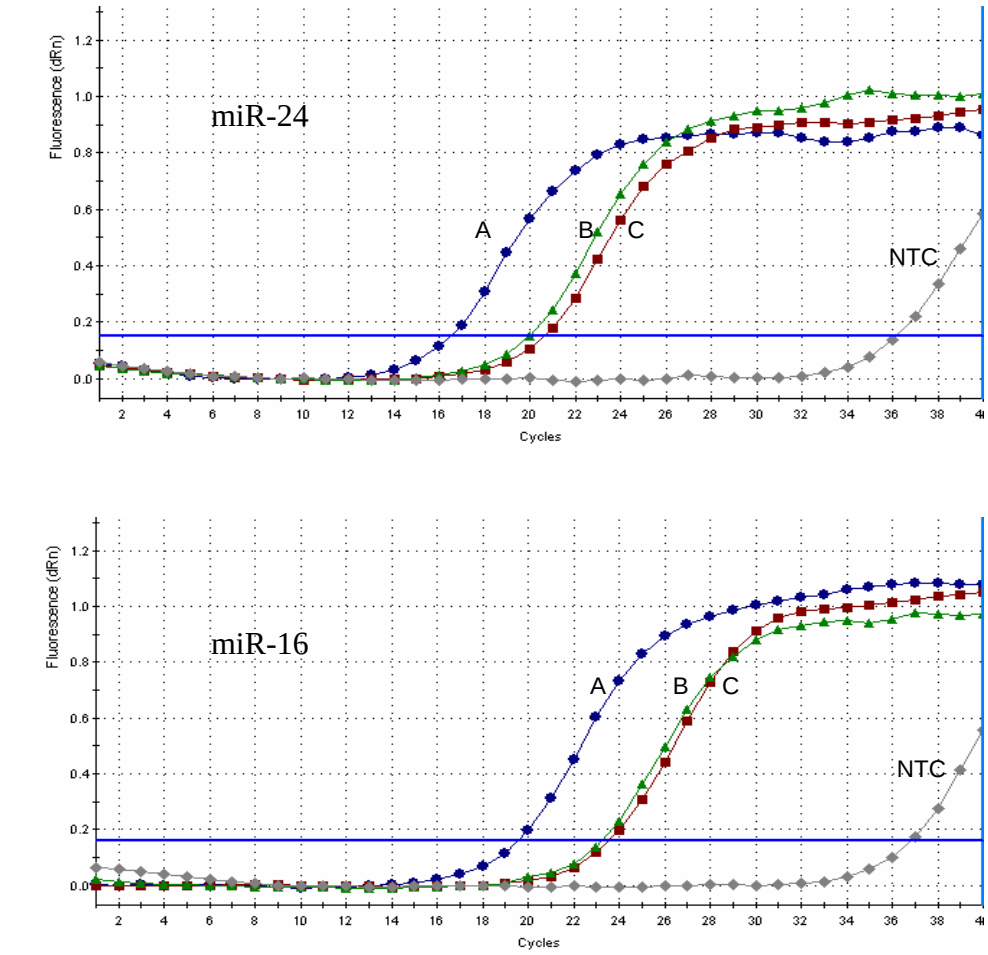
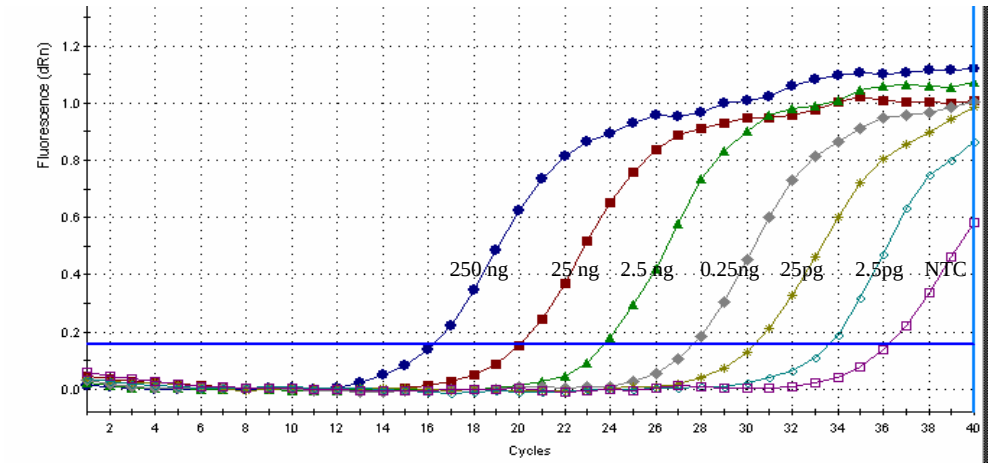


Figure 1: Small RNAs (<200 nt) (25 ng) isolated from human placenta (A), total RNA (25 ng) from human placenta (B) and total RNA (25 ng) from rat lung (C) were analyzed for expression of the indicated miRNA using BioChain's MicroRNA One-Step qRT-PCR Detection Kit and the corresponding primer sets. NTC: no-template control.



$Y=-3.345\text{Log}(x)+30.32$, $R^2=0.997$, Efficiency=99%

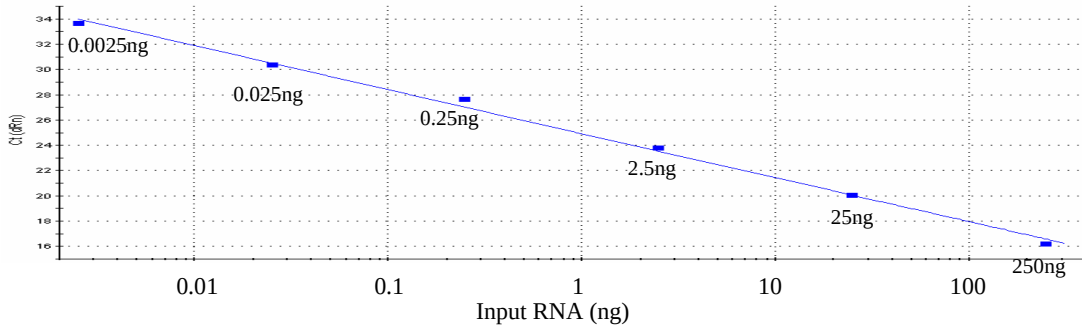


Figure 2. Linear Amplification of miR-24 from a serial dilution of RNA using BioChain's One-Step qRT-PCR MicroRNA Detection Kit. 2.5 pg to 250 ng total RNA isolated from human placenta were used to detect miR-24 using BioChain's microRNA One-Step qRT-PCR Detection Kit and miR-24 primer set. Results demonstrated good linearity of 0.997 and excellent PCR efficiency of 99% over at least 6 logs of dynamic range. This detection system has high sensitivity, detecting miR-24 in as few as 2.5 pg of input total RNA. NTC: no-template control.

Features

- Convenient - All reaction components are supplied for quick and easy set up
- Save time – One-step qRT-PCR procedure reduces setup time and liquid handling steps
- Wide dynamic range: good linearity and excellent PCR efficiency over a 6 logs of dynamic range
- High Sensitivity - detect miRNA expression in as low as 2.5pg total RNA.
- Flexible – Compatible with most of the real-time PCR instruments.

Applications

- MicroRNA detection and quantification
- MicroRNA expression profiling
- MicroRNA array validation

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. Designed for use with BioChain's MicroRNA qRT-PCR primer sets (miR-24 or miR-16 primer set), the MicroRNA One-Step qRT-PCR Detection Kit provides a one-step, simple, robust, inexpensive assay for detection and quantitative analysis of microRNA expression from total RNA samples, or small RNAs enriched samples in DNA with intercalator format.

Quality Control

1 kit of this lot has been tested for amplifying microRNA (miR-24 or miR-16) from total RNA over a 6 logs of dynamic range using Stratagene's Mx3005P as a real time PCR instrument. Good linearity and great PCR efficiency is observed and consistent with the previous lot.

Components

Reagents are sufficient for 200 assays.

miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200)

Item	Amount	Part No.
1. MicroRNA 2x qRT-PCR Reaction Mixture (containing Evagreen Dye)	1.25 ml x 2	KS081200-1
2. MicroRNA qRT-PCR Enzyme Mix*	100 μl	KS081200-2
3. ROX Reference Dye	100 μl	KS081200-3
4. miR-24 qRT-PCR Primer Set (25x)	200 μl	KS081200-4
5. Human Placenta Total RNA (25ng/μl)	100 μl	KS081200-5
6. Nuclease-Free PCR Grade Water	1.75 ml x2	KS081200-6

miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200)

Item	Amount	Part No.
1. MicroRNA 2x qRT-PCR Reaction Mixture (containing Evagreen Dye)	1.25 ml x 2	KS082200-1
2. MicroRNA qRT-PCR Enzyme Mix*	100 μl	KS082200-2
3. ROX Reference Dye	100 μl	KS082200-3
4. miR-16 qRT-PCR Primer Set (25x)	200 μl	KS082200-4
5. Human Placenta Total RNA (25ng/μl)	100 μl	KS082200-5
6. Nuclease-Free PCR Grade Water	1.75 ml x2	KS082200-6

* microRNA qRT-PCR enzyme mix contains Reverse Transcriptase, RNase Inhibitor, and Hotstart Taq DNA Polymerase.

Storage and Stability

Upon receipt, store all components at -20 ºC in a constant temperature freezer. Avoid repeated freeze/thaw cycles. When stored under these conditions this kit is stable for one year after ship date. The EvaGreen Dye in the MicroRNA 2x qRT-PCR Mixture and the ROX reference dye are light sensitive and should be kept away from light whenever possible.

Protocol

(Using Stratagene's Mx3000P™/Mx4000®, and ABI PRISM®/GENEAmp® 5700 Real-time PCR Instrument)

Use of the ROX Reference Dye

ROX reference dye is included in this kit and may be added to compensate for non-PCR related variations in fluorescence. Addition of the reference dye is optional. Optimizing the ROX dye concentration within the qPCR reaction is an important aspect of setup. Too much ROX in the qPCR reaction will reduce background but also makes a low target signal difficult to distinguish from background. Conversely, too little ROX can increase background, meaning that low or weak target signals can be lost. For instruments that allow excitation at ~584 nm (such as Stratagene's Mx instrument and ABI 7500), firstly 1:10 dilute the ROX reference dye provided in the kit, then begin optimization using 0.5 μl **diluted** ROX reference dye in 25 μl qRT-PCR reaction. For instruments that do not allow excitation near 584 nm (such as ABI PRISM®/GENEAmp® 5700 instrument), begin optimization using 0.5 μl **undiluted** ROX reference dye in 25 μl qRT-PCR reaction.

Reagent Preparation and Storage

Thaw the tube containing MicroRNA 2x qRT-PCR Mixture on ice and store it on ice while setting up the reactions. Avoid direct light in preparation of the PCR reaction mixture because EvaGreen dye and ROX reference dye are light sensitive.

1. If the ROX reference dye will be included in the reaction, keep all solutions containing the ROX protected from light.

2. (Optional) Set up a no-template control to screen for contamination of reagents or false amplification.

3. Due to the sensitivity of quantitative PCR, results can be easily affected by pipetting errors. Always prepare a master mix containing the primers and the reference dye (if reference dye is used). Individual pipetting of replicate samples is not recommended.

Real-time PCR Cycling Programs

4. Prepare the following PCR reaction mixture on ice. (First make the master mix without the template. After making the master mix, gently mix the reaction without creating the bubbles, aliquot and then add 1 μl of template to each experimental reaction)

per reaction: 25 μl

Regents	**Volume**
MicroRNA 2x qRT-PCR Reaction Mixture	12.5 μl
MicroRNA qRT-PCR Enzyme Mix	0.5 μl
MicroRNA primer set (25x)	1 μl
Reference Dye ROX [a]	0.5 μl
RNA Template (10-25 ng) or Nuclease-Free Water [b]	X μl
Nuclease-free PCR grade water	Add up to 25 μl

[a] See page 5: Use of the ROX Reference Dye

[b] Optimal amount should be determined by preparing the dilution series. It is recommended to use RNA template in less than 250 ng.

5. Gently mix the reactions without creating bubbles since bubbles interfere with fluorescence detection. Then centrifuge the reactions briefly.

6. Place the reactions in the instrument and run the appropriate PCR program. It is highly recommended to use the following protocol.

   Program for one-step real-time RT-PCR

Cycles	Temp	Time	Detection	Remark
1	16°C	30 min	OFF	This step facilitates RT primer annealing to the RNA template. If the 16°C incubation can not be done at the real-time PCR instrument, this step can be done separately in regular thermal cycler.
2	37°C	30 min	OFF	This step is for the reverse transcription process.
1	95°C	10 min	OFF	This step will inactivate the Reverse Transcriptase and activate the hotstart Taq DNA Polymerase.
40	95°C	15 sec	OFF	Set the instrument to detect and report fluorescence either at the annealing step or the extension step of each cycle.
	37-45°C *	20 sec	ON	
	72°C	30 sec	OFF	

   * Set an appropriate annealing temperature recommended for the primer set used. MicroRNA primer sets were uniquely designed for short length microRNA and optimized to work at low annealing temperature.

MicroRNA qRT-PCR primer set	Recommended PCR Annealing Temp
miR-24	43°C
miR-16	38°C

7. Dissociation Program for microRNA RT-PCR products: Follow manufacturer's guidelines for setting up dissociation depending on the instrument's software version.

End-point PCR

This kit can also be used for end-point PCR. For end-point PCR, amplify for an appropriate number of cycles (usually 20-30 cycles) so that the reaction remains in the exponential phase of amplification, while the PCR amplification product is readily visible on an agarose gel. Analyze the PCR product in 3.5% agarose gel in 1xTAE and stained with Ethidium Bromide or other DNA binding dyes. Gene specific microRNA amplicon is about 60-80 bp.

Related Products

BioChain qRT-PCR ready RNAs, MicroRNA Isolation Kit (Cat# KS 341025). Broad Range Total RNA Isolation Kit for Co-Purifying Large and Small RNAs (Cat# K1341050).

References

1. Liu, J. *et al. Science* 2004. 305: 1437-1441.
2. Tang, G. *Trends Biochem. Sci.* 2005. 30:106-114.