BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

miR-10b One-Step qRT-PCR Detection Kit

Catalog Number: KS083200

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. There is emerging evidence that some microRNAs can function as oncogenes or tumor suppressors. Recent study showed that microRNA-10b (miR-10b) is highly expressed in metastatic breast cancer cells and positively regulates cell migration and invasion. Designed for use with BioChain's MicroRNA qRT-PCR primer sets, the miR-10b One-Step qRT-PCR Detection Kit provides a one-step, simple, robust, inexpensive assay for quantitative analysis of mir-10b expression from total RNA samples, or small RNAs enriched samples. 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 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. Total RNA from metastatic breast cancer cell line MDA-MB-231 is supplied with the kit as positive 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

- Mir-10b detection and quantification
- Breast cancer metastasis studies

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. Mir-10b One-Step qRT-PCR Detection Kit provides a one-step, simple, robust, inexpensive assay for detection and quantitative analysis of mir-10b expression from total RNA samples, or small RNAs enriched samples.

Quality Control

1 kit of this lot has been tested for amplifying miR-10b from total RNA of metastatic breast cancer cell line MDA-MB-231 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-10b One-Step qRT-PCR Detection Kit (Cat# KS083200)

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Item	Amount	Part No.
1. MicroRNA 2x qRT-PCR Reaction Mixture	1.25 ml x 2	KS083200-1
2. MicroRNA qRT-PCR Enzyme Mix*	100 µl	KS083200-2
3. ROX Reference Dye	100 µl	KS083200-3
4. miR-10b qRT-PCR Primer Set (25x)	200 µl	KS083200-4
5. MDA-MB-231 Total RNA (25ng/µl)	100 µl	KS083200-5
6. Nuclease-Free PCR Grade Water	3 ml	KS083200-6

* 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 dye in 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 the dye in the MicroRNA 2x qRT-PCR Mixture 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 the signal fluorescence at the wavelength same to FAM or SybrGreen.
	60°C	45 sec	ON	

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

miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200), miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200), 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. Ma, L. et al. Nature. 2007. 449:682-689.
2. Liu, J. *et al. Science* 2004. 305: 1437-1441.
3. Tang, G. *Trends Biochem. Sci.* 2005. 30:106-114.