Sharpvue™ Human miRNA Primer Array

Performance optimized with, Sharpvue™ miRNA First Strand Kit and Sharpvue™ 2× Universal qPCR Master Mix.

Sharpvue™ Human miRNA Primer Array Set v1.0(384-well)___Cat. No. SW-K1001
Sharpvue™ Human miRNA Primer Array Set v1.0(96-well)____Cat. No. SW-K1007

User Manual Ⅰ

Sharpvue™ Human miRNA Primer Array

Description

Real-Time PCR Technology uses fluorescent material or probes conjugated with fluorophores in the PCR reaction system, and monitors the whole process of PCR reaction by accumulation of fluorescence signal, then qualities and quantities the unknown samples. Compared to chip technology and the common PCR, the Real-Time PCR is fast, precise, quantitative, with high throughput and no chance of contamination, so it is internationally recognized the standard method to quality the nucleosidase molecular.

Sharpvue™ Human miRNA Primer Array use a new non-toxic dye EvaGreen® for detecting, provide a comprehensive solution to your miRNA functional analyses, for both profiling of large numbers of miRNAs and quantitation of individual ones with extremely high sensitivity, specificity, linearity, dynamic range, and reproducibility.

Signalway Biotechnology released most advanced and comprehensive miRNA expression profiling assays, covering 1700 human miRNAs from latest miRBase release of v17.0. and provided two different knids of Arrays as 384-well Array from A to E, 96-well Array from 1to 12. However, You can also order the individual Assay according to your own demands. For a current list of Assays, please visit the website www.Signalway Biotechnologytech.com..

Related Products

Signalway Biotechnology offers comprehensive solutions for studying miRNA and gene

expression. A careful process of codevelop-ment ensures that they work well together and provide robust and reproducible results.

Sharpvue™ miRNA Assay	
Product Name	Description
miRNA RT Kit	High sensitivity and specificity, easy to operate.
Sharpvue™ 2× Universal qPCR Master Mix	Non-toxic EvaGreen-based real-time quantitative PCR Mix
Human miRNA Assay Primer Sets	covering 1700 human miRNAs from latest miRBase release of v17.0, two forms of design are 5 and 20 plates
Human miRNA Primer Array Set v1.0 (384-well)	
Human miRNA Primer Array Set v1.0 (96-well)	
Sharpvue™ Gene Expression Assay	
Gene First Strand Kit	Accurate quantification of mRNA expression
Sharpvue™ 2× Universal qPCR Master Mix (High Rox)	Non-toxic EvaGreen-based real-time quantitative PCR Mix

Contents and Storage

Contents	Quantity	Storage temperature/ conditions
Individual Sharpvue™ Human miRNA Assay Primer Set	3.3×	-20℃,(Stable for at least 12 months) Individual Assay Primer provided as liquid form.
Sharpvue™ Human miRNA Primer Array Set v1.0 (384-well)	A,B,C,D,E 5 plates	-20℃(Stable for at least 12 months), the primer in the every well of the plate provided as powder form..
Sharpvue™ Human miRNA Primer Array Set v1.0 (96-well)	1,2,3,・・・・・19,20 20 plates	

Preparation

Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

IMPORTANT NOTES

PCR arrays require special laboratory practices to avoid false positive amplifications, The high throughput and repetition of these arrays can lead to amplification of a single DNA molecule.

1. All the step ,touch the plate with clean gloves
2.Pick up array plate from the freezer on the desktop at room temperature for 5 minute
3. Centrifuge the plate at 2000 rpm for 2 minute
4.Open the microplate Sealer carefully

Procedure

(This Array Plate can be uesed on ABI7000, 7300, 7700, 7900HT, 7900HT Fast, and StepOnePlus.)

Required Reagents:

Total RNA

Sharpvue™ miRNA First Strand Kit

Forward primer and reverse primer for PCR amplification (customer provide or ordered from Signalway Biotechnology)

Shanrvue™ 2×Universal qPCR Master Mix-High Rox（Cat No: 9000007)

Nuclease Free Water

1. RNA Sample Preparation

This assay starts with total RNA which must includes miRNA. Customers may get purified total RNA with a kit from Qiagen or other manufacturers.

2. Poly(A) Tailing and Reverse Transcription

(a) Set the following components on ice

Add the following reagents into an RNase-free reaction tube which has been pre-cooled on ice. The final volume should be 10μ

Component	Volume(ul)
Total RNA	X
Sharpvue™ miRNA First Strand Kit 5x Mix A （Cat No: 9000005)	2
Sharpvue™ miRNA First Strand Kit 15x Mix B（Cat No: 9000006)	0.67
Nuclease free H_2O (Cat No: 9000016)	to 10
Total	10

(b) Mix gently and spin the tube briefly to collect the contents.

(c) Transfer the tubes to a thermal cycler. Incubate at 37℃for 15 minutes, 25℃ for 15 mins, 37℃ for 30min.

(d) Inactivate the reaction at 85℃ for 5 minutes

(e) Store the single-stranded cDNA at -20℃, or proceed directly to PCR amplification.

3. qPCR

Set the following components：

Component	Volume(ul)
Forward/ Reverse miRNA primer sey (each 3.33×)	3
RT product from step 1	0.67
Shanrvue™ 2× qPCR Master Mix （Cat No: 9000007/10)	5.00
Nuclease Free Water（Cat No: 9000016)	1.33
Total	10.00

Component	Volume(ul)

Forward/ Reverse miRNA primer Array (384 well plate)	0
RT product from step 1	0.67
Shanrvue™ 2× qPCR Master Mix （Cat No: 9000007/10)	5.00
Nuclease Free Water（Cat No: 9000016)	4.33
Total	10.00

Component	Volume(ul)
Forward/ Reverse miRNA primer Array (96 well plate)	0
RT product from step 1	0.67
Shanrvue™ 2× qPCR Master Mix （Cat No: 9000007/10)	5.00
Nuclease Free Water（Cat No: 9000016)	4.33
Total	10.00

(b) Seal the plates with qPCR film and mix gently, then spin the tube briefly to collect the contents.
(c) Transfer the plate to a real time thermal cycler (e.g. ABI 7900)
(d) Set the thermal profile as follows:
4. Set the one of three kinds of thermal profile as follows or followed manual instruction of Manufacture :
Set: Detector as SYBR, Passive Reference as ROX
i. Two-step fast cycling protocol

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme hot activation	96℃	2 minutes	1
Denaturation	96℃	5 seconds	40
Annealing & Extension	60℃	30 seconds	

ii. Three-step fast cycling protocol

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme hot activation	96℃	2 minutes	1
Denaturation	96℃	5 seconds	40
Annealing & Extension	60℃	30 seconds	

Cycling Step	**Temperature**	**HoldingTime**	**Number of Cycles**
Enzyme hot activation	96℃	2 minutes	1
Denaturation	96℃	15 seconds	40
Annealing & Extension	60℃	60 seconds	

5. Dissociation Curve Measurement (optional), default program in ABI 7900.
96℃ for 15 seconds, 60℃ for 15 seconds，96℃ for 15 seconds
6. Analyze the experiment
Refer to the getting started guides for your real-time PCR system to analyze the experiment. The general process for analyzing the data from gene expression assays involves the following procedures:
a. View the amplification plots
b. Set the baseline and threshold values.

Example:
Objective: let-7 family
Equipment: ABI 7900
Key Product Features
Universal RT primer to minimize variations in RT step.
Highly specific-only measure miRNAS not precursor miRNAS. The proprietary primer design of the Sharpvue™ miRNA Arrays and Assays.
Distinguishes miRNA family members with single nucleotide mismatches(e.g.let-7 family members only have less than 4% cross-reactivity).
Highly sensitivity-detects miRNA in single molecules with 0.995 of linear range, and 6 orders of dynamic range.
Sensitivity and Linearity of Sharpvue™ miRNA Assays

Figure 1 demonstrated Sharpvue™ miRNA assays have high sensitivity, detecting single miRNA copy. Sharpvue™ miRNA assays have excellent linearity with R_2>0,99 and wide dynamic range>6 log

Specificity of Sharpvue™ miRNA Assays

Trouble Shooting Guide

Poor precision or failed qPCR reactions	• The fluorescence detection temperature may not be appropriate. Adjust accordingly. • The set up position for reaction samples in the real-time PCR instrument may not be right. A djust accordingly. • PCR cycle conditions, primer concentration and primer sequences may not be appropriate. Adjust the primer concentration and annealing temperature.If this does not work,redesign the primers. • The template sample purity may not be adequate. Purify the template sample by phenol/chloroform extraction and ethanol precipitation. If the samples are reverse transcribed cDNA, set up the qPCR reaction with a diluted sample as other concentrated reagents in the RT reaction mixture may be interfering with the qPCR. • Try to use 0.3% agarose gel electrophoresis to check the qPCR products. Check the purity of the primers by electrophoresis or use PAGE-purified primers if the bands are diffused. One may also use phenol/chloroform extraction and ethanol precipitation methods to treat the primers before the experiment.
Abnormal melting curves	**Signal in the blank(No Template Control) sample** • There may be contamination of the positive samples in the qPCR reaction system if the Tm of the melting curve of the blank control is the same as the positive control.Eliminate sample application error first. If the situation still persists, replace the PCR grade water and/or primers and/or use a new Sharpvue™ 2× Universal qPCR Master Mix (High Rox). • If the Tm of the melting curve of the blank control is lower than the positive control, the qPCR reaction may have produced nonspecific amplification such as primer-dimers. Prepare the qPCR reaction mix on ice and increase the temperature of fluorescence detection. If this does not work, redesign the primers.

	Double peaks and multiple peaks in the melting curve of the positive control • In the absence of other primers present in the reaction, double or multiple peaks in the melting curve of the positive control indicate that the qPCR reaction produced nonspecific amplification fragments. Prepare the qPCR reaction mix on ice; optimize the qPCR reaction conditions, for example, by increasing the annealing temperature, decreasing the primer concentration or increasing the fluorescence detection temperature (not more than the Tm value of the expected product). If this does not work, redesign the forward primer.
No signal (Ct) or late appearing signal	• Not enough PCR cycles. For good sensitivity, one should generally set up more than 35 PCR cycles, but more than 45 cycles may result in too much background signal. • The amount of template used may not be enough or the template may be degraded. Use the highest concentration possible of diluted template samples to set up the qPCR. At the same time, avoid freezing and thawing the samples repeatedly. • The amplification efficiency is low and the qPCR reaction conditions are not optimal. Redesign the primers and optimize the reaction conditions.

Limited Use License and Warranty

Following terms and conditions apply to use of all miRNAs and Packaging Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to Signalway Biotechnology within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from Signalway Biotechnology. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research . Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

Signalway Biotechnology warrants that the Product meets the specification described in the accompanying Product Datasheet .If it is proven to the satisfaction of Signalway Biotechnology that the Product fails to meet these specifications , Signalway Biotechnology will replace the Product. In the event a replacement cannot be provided, Signalway Biotechnology will provide the purchaser with a refund . This limited warranty shall not extend to anyone other than the original purchaser of the Product . Notice of nonconforming products must be made to Signalway Biotechnology 30 days of receipt of the Product. Signalway Biotechnology's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price . Signalway Biotechnology's liabilitydoes not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. Signalway Biotechnology does not provide any other warranties of any kind , expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

Signalway Biotechnology is committed to providing our customers with high-quality products . If you should have any questions or concerns about any Signalway Biotechnology products, please contact us at 866-998-6722.

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