盛元生物
Biovue Technology

Sharpvue™ miRNA First Strand Kit

For reliable first-strand cDNA synthesis from all miRNA sources

Cat. No.9000004

(25 reactions)

User Manual Ⅰ

Sharpvue™ miRNA First Strand Kit

Sharpvue™ miRNA Assay is the lasted product from Biovue. It is an EvaGreen® dye-based real time qPCR method for specific and quantitative detection of mirco RNA.

Description

Comparing with similar products from other international manufacturers on the market, Biovue boasts (1) the largest collection of validated Assays (>1700 human miRNAs in miRBase v 17.0), (2) one of the largest detection range (up to 6 magnitude, as shown in Figure 1), (3) the most sensitive detection (down to a single copy number, as shown in Figure 1), (4) the highest specificity (less than 3% cross-reactivity among 8 members of let7 family, which is almost half of the second best performer in the market today), and (5) equal or almost equal efficiency in amplification of each individual miRNA .

This assay starts with total RNA which must includes miRNA. Customers may get purified total RNA with a kit from Qiagen or other manufacturers. In the first step, by using Sharpvue™ miRNA First Strand Kit, poly (A) tails are added to the whole population of miRNA, and immediately, the tailed miRNA population is converted into a population of cDNA from the RT primer. Next, a particular species of derived cDNA is quantified and differentiated from the rest in the next qPCR by using forward primer, reverse primer and 2X Sharpvue™ qPCR Master Mix （Cat No: 9000007）.

Proprietary primer design and its combination with the use of Sharpvue™ qPCR Master Mix assure extraordinary sensitivity, specificity and equal efficiency. Sharpvue™ qPCR Master Mix uses our proprietary DNA polymerase, microTaq. This enzyme remains inactive in the master mix, until after 2 minutes at 95 ℃. The active form of microTaq is so efficient in amplifying small amplicons that LNA-containing primers are not needed.

Related Products

Sharpvue™ miRNA Assay	
Product Name	Description
miRNA RT Kit	High sensitivity and specificity, easy to operate.
Sharpvue™ 2× Universal qPCR Master Mix (High Rox)	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-toxicEvaGreen-based real-time quantitative PCR Mix

Contents and Storage

Contents	Quantity	Storage temperature/ conditions
5× MixA	25 reacction	-20℃
15 ×MixB	25 reacction	-20℃
dd H_2O (RNase and DNase free)	1ml	Room temperature

Preparation

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

chemicals.

RNA Sample Preparation

When working with RNA it is important to avoid RNases in your solutions,consumables and labware. When preparing your RNA samples,always wear a mask and disposable gloves in all procedures. Follow the described procedures you are using for RNA extraction carefully. Ready-to-use solutions that are RNase –free can be purchased. Alternatively treat solutions with diethyl pyrocarbonate (DEPC) and then autoclave. RNases on labware can also be inactivated by DEPC treatment or by baking at 250℃ for 3 hours. Use DEPC to treat all microcentrifuge tubes,pipettes tips (if not RNase free) and then autoclave to deactivate RNase. RNase-free consumable are available for purchase from many commercial sources.

IMPORTANT NOTES

1. Store kit at -20℃. Avoid storage or leaving reagents at 4℃ or room temperature.
2. Touch gently to avoid shocking severely,then briefly centrifugr before use.
3. Set up all reactions on ice to reduce risk of RNA degradation.
4. Read all procedures before setting up RT reaction.

Procedure

1. 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 10μl

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

* For Multiple reactions, prepare a master mix of common components .

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

(c) Transfer the tubes to a thermal cycler. Incubate at 37℃for 60 minutes.

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

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

2. qPCR。

(a) Set the following components：

Component	Volume(ul)
Forward/ Reverse miRNA primer set (each 3.33x)	3.00
RT product from step 1	0.67
2X Shanrvue™ qPCR Master Mix-High Rox（Cat No: 9000007)	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
2X Shanrvue™ qPCR Master Mix-High Rox（Cat No: 9000007)	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
2X Shanrvue™ qPCR Master Mix-High Rox（Cat No: 9000007)	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:

Cycling Step	Temperature	HoldingTime	Number of Cycles
Enzyme hot activation	96℃	2 minutes	1
Denaturation	96℃	5 seconds	40
Annealing	60℃	30 seconds	
Melting analysis	96℃	15 seconds	1
	60℃	15 seconds	
	96℃	15 seconds	

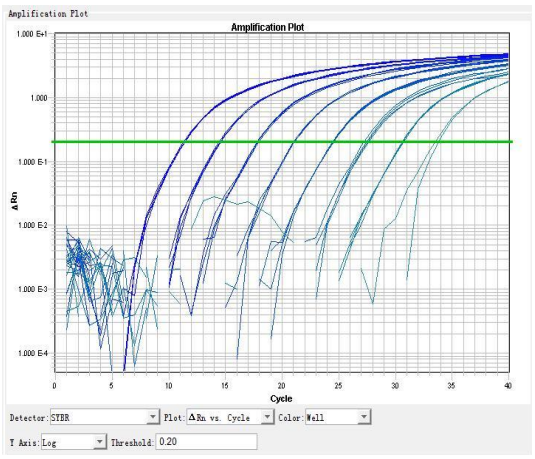
Set the optical parameters as follows:

Reporter: SYBR or FAM

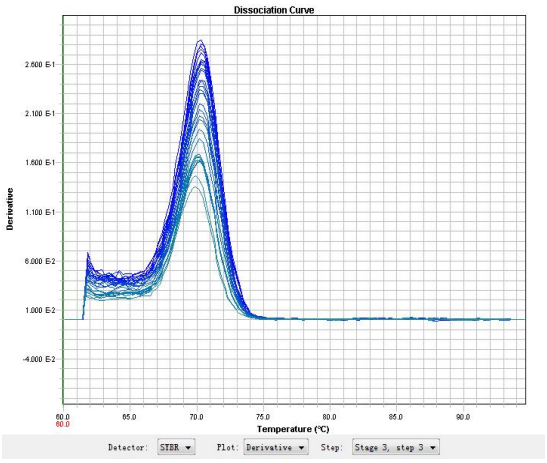
Passive reference (for some instrument) : ROX

Example

The amplification efficiency and detection sensitivity of the Sharpvue™ miRNA First Strand Kit are assessed by standard curves made by gradient dilution of the miRNA let-7a.



Amplification curves of serially diluted miRNA 7a



Peak values of amplified products in melting curves

The peak values from the amplification and melting curves show that as low as 0.001 pM can be detected

when using miRNA 7a as a template and that there is only a single amplified product, showing that very high sensitivity can be attained using the Sharpvue™ miRNA First Strand Kit.

Trouble Shooting Guide

Little for no RT-PCR product

RNA template degradation

The quality of RNA is the key factor for cDNA synthesis. Follow the RNA isolation kit procedure carefully, always wearing a lab coat, gloves and maskwhen working with RNA and use RNA-Grade reagents and materials. Check the RNA quality by RNA electrophoresis in a denaturing gel.

An inhibitor was present in the RNA template

Trace amounts of inhibitor such as guanidine salts in the RNA template can inhibit the cDNA synthesis. Re-precipitate the RNA with ethanol and wash the pellet with 75% ethanol.

A G-C rich template or secondary structure of the amplification product is obstructing the reaction

Prepare the RNA-Primer Mix before the RT step. Then add a PCR enhancing reagent such as DMSO, betaine, etc. in the PCR reaction.

PCR product is longer than expected

Genomic DNA was present. Perform a DNase Ⅰdigest before the RT step or design intron-spanning or flanking primers to avoid co-amplification of genomic DNA.

The wrong product was amplified. Optimize the PCR reaction conditions.

Limited Use License and Warranty

Limited Use License

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 Biovue 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 Biovue. 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

Biovue warrants that the Product meets the specification described in the accompanying Product Datasheet .If it is proven to the satisfaction of Biovue that the Product fails to meet these specifications , Biovue will replace the Product. In the event a replacement cannot be provided, Biovue 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 Biovue 30 days of receipt of the Product. Biovue's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price . Biovue'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.

Biovue does not provide any other warranties of any kind , expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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