盛元生物
Biovue Technology

Sharpvue™ Gene First Strand Kit

For reliable first-strand cDNA synthesis from all mRNA sources

Cat. No.9000001

(25 reactions)

User Manual Ⅰ

Sharpvue™ miRNA First Strand Kit

Sharpvue™ Gene Expression Assay, an EvaGreen®-based real time quantitative PCR method for gene expression detection.

Description

Biovue high-performing gene expression assay is based on Biovue's proprietary microTaq DNA polymerase and EvaGreen® dye. MicroTaq DNA polymerase is inactive in its original form and become highly active and highly specific after two-minute incubation at 95 ^{o}C. This assay has several advantages. First, primer dimer formation is greatly diminished. Second, reactions could be accomplished very quickly, each cycle may just take less than 30 seconds. Assay could be done with 2-tube protocol. Unique among the similar products on the market, our assay system employs EvaGreen®. After cycling, the amplicon could be subjected to high definition melting analysis, giving researchers more definitive answer to the purity of their product. Also, the high definition melt could be used to detection SNP or mutation in expressed mRNA.

Biovue respects researchers, human kind and our environment. All components in our Sharpvue™ Gene Expression Assay system are friendly to human and environment. Neither mutagens nor cytotoxic agents are used.

Related Products

<table>
<tr><td colspan="2">Sharpvue™ miRNA Assay</td></tr>
<tr><td>Product Name</td><td>Description</td></tr>
<tr><td>miRNA RT Kit</td><td>High sensitivity and specificity,
easy to operate.</td></tr>
<tr><td>Sharpvue™ 2× Universal qPCR Master Mix (High Rox)</td><td>Non-toxic EvaGreen-based
real-time quantitative PCR Mix</td></tr>
<tr><td>Human miRNA Assay Primer Sets</td><td rowspan="3">covering 1700 human miRNAs from latest miRBase release of v17.0, two forms of design are 5 and 20 plates</td></tr>
<tr><td>Human miRNA Primer Array Set v1.0 (384-well)</td></tr>
<tr><td>Human miRNA Primer Array Set v1.0 (96-well)</td></tr>
<tr><td colspan="2">Sharpvue™ Gene Expression Assay</td></tr>
<tr><td>Gene First Strand Kit</td><td>Accurate quantification of
mRNA expression</td></tr>
<tr><td>Sharpvue™ 2× Universal qPCR Master Mix (High Rox)</td><td>Non-toxic EvaGreen-based
real-time quantitative PCR Mix</td></tr>
</table>

Contents and Storage

Contents	Quantity	Storage temperature/ conditions
5× MixA	25 reacction	-20℃
40×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. Reverse transcription of mRNAs into single-stranded cDNAs by using Sharpvue™ Gene First Strand Kit

(a) Set the following components on ice in a tube

Component	Volume (μl)
RNA sample	X
Sharpvue™ Gene First Strand Kit 5x Mix A (Cat No: 9000002)	2
Sharpvue™ Gene First Strand Kit 40x Mix B (Cat No: 9000003)	0.25
Sharpvue™ Nuclease Free Water (Cat No: 9000016)	to 10

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

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

(c) Transfer the tube to a thermal cycler. Incubate at 42℃ for 5- 60 minutes, then incubate at 85℃for 5 minutes to inactivate the reaction.

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

2. Quantifying cDNAs with real-time PCR by using forward primer, reverse primer and 2X Sharpvue™ qPCR Master Mix

(a) Set the following components in a tube or in a PCR plate：

Components	Volume (μl)
Forward/Reverse primer mixture (each 3.33x)	3
RT product from (d)	0.67
Sharpvue™ 2x Universal qPCR Master Mix (Cat No: 9000007)	5
Add Nuclease free H_2O	to 10

* Reaction volume could be proportionally changed to 5 to 50 μl.

(b) Cap the tube or seal the plate with optical film. Mix gently, then spin the tube briefly to collect the contents.

(c) 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℃	20-60 seconds	
Melting analysis	96℃	15 seconds	1
	60℃	1 minutes	
	96℃	15 seconds	

(d) Transfer the tube or the plate to a real time thermal cycler.

3. Real time measurement of the fluorescence change of EvaGreen® fluorescent dye.

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

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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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