ShineProbe® Real Time qPCR MasterMix Kits User Manual

Cat.Nos.ZK00713(50 rxns×50ul)

ZK00714(100 rxns×50ul)

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Ⅰ、Description

ShineProbe is a 2X convenient premix reagent, specially designed for real time PCR by using various detection probes (eg. TaqMan, Molecular Beacon, etc.) ，but not suitable for sybr green.The master mix contains all reagents needed for qPCR. Only template ,Probe,and PCR primers need to be added by the user. The ROX dye is not provided, For most real-time instruments ROX passive reference dye is not required .This product combines the high performance of *Taq*, which is an enzyme for hot start PCR utilizing Taq antibody, with a newly developed buffer which provides superior specificity, increased amplification efficiency and high aptitude for high-speed real time PCR. Accordingly, a successful real time PCR is promised with high sensitivity, wide dynamic range and accurate quantification. The reaction chemistry of ShineProbe qPCR Kit is applicable to most real-time PCR instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, Roche,and Stratagene.

Ⅱ、Component

ZK00713	50 reactions X 50ul each	Hotstart fluo-PCR mix (2 X conc.)
ZK00714	100 reactions X 50ul each	Hotstart fluo-PCR mix (2 X conc.)

Ⅲ、Shipping and Storage

The ShineProbe qPCR Kit is shipped in dry ice or ice bag. Upon arrival, store all kit components at -20°C. When using the 2x master mix, the leftover thawed mix can be refrozen and stored at -20 °C without affecting the performance of the kit. The kit is stable for six months from the date of packaging when stored and handled properly.

Ⅳ、Reaction Setup

Components	Volume(μl)	Final conc.	Comments
Hotstart Fluo-PCR mix	25	1 X	Mix thoroughly
Sense primer(25pmol/ul)	1	0.3~1uM	Titrate from 0.3 to 1 μM if necessary.
Anti-sense primer(25pmol/ul)	1	0.3~1uM	Titrate from 0.3 to 1 μM if necessary.

Probe(25pmol/ul)	0.5	0.15~0.5uM	
cDNA(ul)	2	10~100ng	
ddH_2O (ul)	20.5	-	Add water to fill up to the final reaction volum
Total Volume(ul)	50	-	

Ⅴ、Cycling Protocol

1、Protocol using LightCycler with Taqman probe

93℃ 2min→93℃5 sec→60℃30 sec
└──────┘
40cyclers

2、Protocol using LightCycler with Molecular Beacon probe

93℃ 2min→93℃5 sec→60℃20 sec→72℃20 sec
└──────────────┘
40cyclers

3、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Taqman probe

94℃ 4min→94℃ 30sec→60℃ 60sec
└──────┘
40cyclers

4、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Molecular Beacon probe

94℃ 4min→94℃ 30sec→60℃ 30sec→72℃30sec
└──────────────┘
40cyclers

Ⅵ、Reference

1.Chou, Q., Russell, M., Birch, D., Raymond, J. & Bloch, W. (1992) Prevention of pre-PCR mispriming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res.*20:1717–1723.

2.Roux, K. H. (1995) Optimization and troubleshooting in PCR. *PCR Methods Appl.* 4:5185–5194.

3.Sambrook, J. & D. W. Russell. (2001) Molecular Cloning: A Laboratory Manual, Third Edition (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

4.Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(t)) Method.*Methods* 2001, 25:402-408.

5.L D Ke, Z Chen .A reliability test of standard-based quantitative PCR: exogenous vs endogenous standards Mol Cell Probes. 2000 Apr;14(2):127-35.

6.Weihong Liu and David A. Saint Validation of a quantitative method for real time PCR kinetics Biochemical and Biophysical Research Communications 294 (2002) 347–353

Ⅶ、House-keeping genes

When studying gene expression, the quantity of the target gene transcript needs to be normalized against the quantity of a house-keeping gene transcript in the same sample. A house-keeping gene or several genes are used to normalize data against variation in sample quality and quantity between samples. Examples of commonly used house-keeping genes are GAPDH,beta actin and 18S rRNA. A gene used as a reference should have a constant expression level independent of the variation in the state of the sample tissue. A problem is that even with housekeeping genes the expression usually varies to some extent. That is why several house-keeping genes are usually required, and their expression needs to be checked for each experiment.

Ⅷ、Relative quantification

Relative quantification is used to determine the ratio between the quantity of a target molecule in a sample and in the calibrator (calibrator being e.g. healthy tissue or untreated cells). The most common application of this method is the analysis of gene expression, e.g. comparisons of gene expression levels in different samples. Target molecule quantity is usually normalized with a house-keeping gene.Comparative C(t) method can be used for relative quantification. Both the sample and the calibrator data is first normalized against variation in sample quality and quantity. Normalized values, C(t)s, are first calculated from following equations:

$\Delta C(t)_{sample} = C(t)_{target} - C(t)_{house\text{-}keeping\ gene}$

$\Delta C(t)_{calibrator} = C(t)_{target} - C(t)_{house\text{-}keeping\ gene}$

The $\Delta\Delta C(t)$ is then determined using the following formula:

$\Delta\Delta C(t) = \Delta C(t)_{sample} - \Delta C(t)_{calibrator}$

Expression of the target gene normalized to the house-keeping gene and relative to the calibrator= $2^{-\Delta\Delta C(t)}$.

Ⅸ、Troubleshooting

No fluorescence signal at all	
Possible causes	**Comments and suggestions**
Error in cycler setup	Check that instrument settings correspond with the experiment.
Missing components (e.g. primers, probe or template)	Check the assembly of the reaction.
Probe is not labelled very well.	Re-label probe.
Missing essential step in the cycler protocol	Check the cycler protocol.
Sample configured as empty	Check the plate configuration.
Late increase in fluorescence signal	
Possible causes	**Comments and suggestions**
Error in cycler setup	Check that instrument settings correspond with the experiment.
Insufficient starting template	Check the calculation of template stock concentration; increase template amount if possible.
Annealing temperature too high	Use gradient to optimize annealing temperature; Decrease annealing temperature in 2° C decrements if a gradient feature is not available.
Probe is not labelled very well.	Re-label probe.
Insufficient extension time for the amplicon size	Increase extension time.
Primer or probe concentration too low	Increase primer concentration (to max 1000 nM each). 250 nM probe concentration is usually sufficient.
PCR protocol not optimal	Make sure the recommended PCR protocol is used. If necessary,optimize using the recommended protocol as a starting point.
Normal fluorescence signal, but low efficiency	
Possible causes	**Comments and suggestions**
Pipetting error	Check the assembly of the reactions.
Primer – dimers from previous run contaminating the reaction	Perform UNG treatment prior to PCR cycling.
Primer and probe design not optimal or very low template concentration	Re-check primer and probe design and template stock concentration.
Probe is not labelled very well.	Re-label probe.
Inhibitors from the sample affecting reaction	Repurify DNA.
Low initial template concentration	Increase template amount.

Non-linear correlation between C(t) and log of template amount in standard curve	
Possible causes	**Comments and suggestions**
Template dilution inaccurate	Remake dilution series and make sure the samples are well mixed.
Template amount too high	Reduce the template amount; Increase reaction volume.
Template amount too low	Increase template amount.
Primer-dimers co-amplified	Redesign primers.

Ⅹ、Importants Notes

1、User can adjust annealing temperature and time according as the Tm of special primer and probe.

2、Reaction volume from 20 to 50 µl is recommended for most real-time instruments. If using Lightcycler,the reaction volume can reduce to 20ul.

3、The temperature should be optimized within the range of 55-66℃ if optimization is required. When reaction does not proceed efficiently, extend the time or change the reaction from 2 step PCR.to 3 step PCR or from 3 step PCR to 2 step PCR.

4、For Research Use Only. Not for use in diagnostic procedures.

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Ⅺ、Technical Supports

To access the ShineGene Web site, go to:**http://www.shinegene.org.cn**

The ShineGene Web site provides a list of telephone,Email and fax numbers that can be used to contact Technical Support. E-mail:master@shinegene.org.cn

Ⅻ、Related Products

ShinePrep® RNA Miniprep Kits

Cat.Nos.ZN00701(50 rxns)

ZK00702(100 rxns)

PowerQ® Taq plymerase

Cat.Nos.ZP00102(1000 U)

ZP00103(5000 U)

FicoScript® M MLV Reverse Transcriptase

Cat.Nos.ZP00601(1000 U)

ZP00602(5000 U)

ShinePolo® One step RT-PCR qPCR Kits

Cat.Nos.ZK00102(50 rxns×30ul)

ZK00103(100 rxns×30ul)

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