InRcute lncRNA qPCR Kit (SYBR Green)

Sensitive and stress-resistant special fluorescent quantitative reagent for lncRNA

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lnRcute lncRNA qPCR Kit (SYBR Green)

Cat. no. GFP402

Kit Contents

Contents	GFP402-02 20 μl×500 rxn
2×lnR lncRNA PreMix (SYBR Green)	4×1.25 ml
50× ROX Reference Dye	1 ml
RNase-Free ddH_2O	5×1 ml
Handbook	1

Storage

Please store the product at -30~-15°C immediately after receiving it. When taking out from -30~-15°C for use, dissolve the frozen 2× lnR lncRNA PreMix and ROX Reference Dye, and then mix them gently by inverting up and down until the solution is completely uniform before use. If it is not used after thawing, it must be thoroughly mixed and then frozen again (salt will be delaminated during thawing; if it is not mixed before freezing, the precipitation of salt crystals will cause damage to enzymes). If it needs to be used frequently for a period of time, it can be stored at 2-8°C for 3 months. Avoid repeated freezing and thawing. This product can be stored at -30~-15°C for 2 years.

Introduction

The kit adopts the principle of SYBR Green I chimeric fluorescence to carry out fluorescence quantitative detection of lncRNA. The kit contains all reagents for quantitative detection of lncRNA, including 2× lnR lncRNA PreMix, 50× ROX Reference Dye and RNase-Free ddH_2O. The target lncRNA can be rapidly and specifically quantitatified by this kit.

Compared with mRNA, lncRNA has the characteristics of low abundance, large difference in GC content and more complex secondary structure. Traditional quantitative reagents are difficult to achieve ideal effects on lncRNA. The 2× lnR lncRNA PreMix in this kit is a new generation of pre-mixed fluorescent quantitative PCR detection reagent specially developed for the quantitative detection of lncRNA. The DNA Polymerase in this kit is an antibody modified hotstart enzyme, and matching with a specially optimized buffer system, it can ensure that the product has higher detection sensitivity while ensuring higher reaction specificity. In addition, the addition of H-competitor factor and EP component in the buffer system ensures a wide range of sample universality, and has very good amplification applicability for templates with different GC contents, templates with complex advanced structures, templates with more PCR inhibitor residues and long fragment templates, etc. It is especially suitable for quantitative detection of lncRNA with relatively complex advanced structures and relatively low overall abundance. In addition, the optimized premix can shorten the reaction time of Real-Time PCR and is suitable for standard or rapid quantitative PCR reactions.

Features

1. **Easy operation:** SYBR Green I is pre-mixed in 2× lnR lncRNA PreMix. When preparing PCR reaction solution, simply add template, primers and sterilized distilled water to carry out fast Real-Time PCR reaction, which is simple and convenient to operate.
2. **Good universality:** The product contains H-Competitor factor, which can compete for hydrogen bonds and enhance the opening strength of double strands, so that the product has wide sample universality, and has superior applicability to templates with complex advanced structures, long fragment amplification, etc.
3. **Excellent performance:** The special fast PCR Buffer system of this product contains a unique PCR stabilizing factor-EP, which can effectively protect the enzyme activity and resist the interference of various PCR inhibitors, ensuring that 2× lnR lncRNA PreMix has the characteristics of high amplification efficiency, high amplification specificity, high amplification sensitivity and wide credible range.

4. **Accurate quantification:** The ROX Reference Dye supplied in this product can be used to eliminate the signal background and to correct the error of fluorescence signal generated between wells, and is convenient for users to select corresponding concentrations for different types of fluorescence quantitative PCR instruments.
5. **Good stability:** 2× lnR lncRNA PreMix is packed in colorless transparent tube. After detection, light will not affect the quantitative results of the system.

Precautions <u>Please carefully read these precausions before use.</u>

1. If the reagents are not mixed evenly, the reaction performance will be decreased. When using, please mix it by gently inverting up and down. Please do not mix it evenly with vortex, try to avoid foam, and use it after instantaneous centrifugation.
2. Primer purity has a great influence on reaction specificity, so it is recommended to use primers purified above PAGE level.
3. In the 20 μl reaction system, the amount of cDNA template used is generally less than 100 ng, and the amount of genomic DNA template is generally less than 50 ng. When the reverse transcription product is used as the template, the amount used should not exceed 10% of the final volume of PCR system.

Protocol

<1> Establish Real-Time PCR reaction system

Please note that 50× ROX Reference Dye should be kept away from light.

1. Thaw 2× lnR lncRNA PreMix (if stored at -30~-15°C), ROX Reference Dye, templates, primers and RNase-Free ddH_2O at room temperature and thoroughly mix all the reagents.
2. It is suggested to prepare Real-Time PCR reaction solution on ice.

Reaction system:

Components	50 μl system	25 μl system	20 μl system	Final concentration
2× lnR lncRNA PreMix	25 μl	12.5 μl	10 μl	1×
Forward primer (10 μM)	1.25 μl	0.625 μl	0.5 μl	0.25 μM*
Reverse primer (10 μM)	1.25 μl	0.625 μl	0.5 μl	0.25 μM*
cDNA template	-	-	-	-ng-pg
50× ROX Reference Dye△	-	-	-	-
RNase-Free ddH_2O	To 50 μl	To 25 μl	To 20 μl	-

* Primer with the final concentration of 0.25 μM can obtain good amplification results in most systems. When the amplification efficiency is not high, the primer concentration in the PCR reaction system can be increased. When non-specific amplification occurs, the primer concentration in the PCR reaction system can be appropriately reduced. If the concentration of primer needs to be further optimized, it can be adjusted in the range of 0.2-0.5 μM.

△ The optimum ROX Reference Dye concentrations for several common instruments are shown in the following table:

Instrument	Final concentration
ABI 5700/7000/7300/7700/ 7900HT/StepOne™/StepOne Plus™	5x (e.g. 5 ul ROX/ 50 μl system)
ABI 7500/7500 Fast/ViiA 7/ QuantStudio™/12K Flex; Agilent Mx3000P/Mx3005P/ Mx4000	1x (e.g. 1 μl ROX/ 50 μl system)
TGreat Real 96, Roche instruments, Bio-Rad instruments, Eppendorf instruments, etc.	No need

<2> Run Real-Time PCR program

It is suggested to adopt a two-step PCR reaction procedure for the reaction. If the amplification effect is poor due to low template quantity and other factors, a three-step procedure can be used for PCR reaction.

Two-step reaction program:

Phase	Cycle	Temperature	Time	Content	Fluorescence signal acquisition
Initial denaturation	1×	95°C	3 min	Initial denaturation	No
PCR reaction	40×	95°C	5 sec	Denaturation	No
		60°C △1	15 sec △2	Annealing/ Extension	Yes
Melting/Dissociation Curve Stage					

Three-step reaction program:

Phase	Cycle	Temperature	Time	Content	Fluorescence signal acquisition
Initial denaturation	1×	95°C	3 min	Initial denaturation	No
PCR reaction	40×	95°C	5 sec	Denaturation	No
		50-60°C △3	10 sec	Annealing	No
		72°C	15 sec △2	Extension	Yes
Melting/Dissociation Curve Stage					

△1 First use 60°C 15 sec for amplification. If further optimization is needed, it can be optimized in the range of 56-66°C.

△2 When using different types of instruments to set the time, please carry out the experimental operation according to the requirements of the instrument operating instructions. See the following table for the time setting of several common instruments:

When using TGreat Real 96, ABI 7700/7900HT/7500 Fast/ViiA 7, Roche, BioRad and Agilent fluorescence quantitative PCR instruments, please set it at 15 sec.
Set ABI 7000 and 7300 at 31 sec.
Set ABI 7500 at 32 sec.

△3 Usually, the annealing temperature of the primer is 5°C lower than the melting temperature (Tm) of the primer. If the base number of primer is small, the annealing temperature can be appropriately increased, which can increase the specificity of PCR. If the number of bases is large, the annealing temperature can be appropriately reduced.

3. Cover the reaction tube and mix gently. Centrifuge briefly to ensure that all components are at the bottom of the tube.
4. Place the reaction tubes in the real-time PCR instrument, and run the program.