UltraSYBR Mixture

Item No. U665891 (5 mL)
U665891 (40 mL)
Storage condition: -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

individual parts making up a compound	U665891 5 ml	U665891 40 ml
2×UltraSYBR Mixture	5 x 1 ml	40 x 1 ml
ddH2O	5 x 1 ml	40 x 1 ml

Product Introduction

UltraSYBR Mixture is a premixed system for dye-based (SYBR Green I) real-time fluorescent quantitative PCR. It contains GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, and Mg2+ at a concentration of 2×, which is easy to operate. It is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.
The fluorescent dye SYBR Green I can bind to all double-stranded DNAs, which makes it possible to detect different target sequences without the need to synthesize specific labeling probes. GoldStar Taq DNA Polymerase is a chemically modified, new and highly efficient hot-start enzyme, which has no polymerase activity at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of the primer to the template or primer dimerization at room temperature, and the enzyme activation requires 10 minutes of incubation at 95° C. The unique PCR buffer system and hot-start enzyme can be used for the detection of different target sequences without the need to synthesize specific labeling probes. The unique combination of PCR buffer system and hot start enzyme effectively inhibits non-specific PCR amplification and significantly improves PCR amplification efficiency. It is suitable for use with fluorescent PCR instruments that do not require ROX as a correction dye, such as the Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, and CFX96.

Product Features

1. This product uses a new high-efficiency hot-start enzyme GoldStar Taq DNA Polymerase and a unique PCR buffer system, which significantly improves the PCR amplification efficiency, with high sensitivity and specificity.
2. Suitable for fluorescence quantitative PCR assay, which can accurately quantify and detect the target gene.

Caveat

1. Before use, please mix gently by turning up and down, avoid foaming as much as possible, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye. Avoid strong light when storing this product or preparing PCR reaction solution.
3. Avoid repeated freezing and thawing of the product, repeated freezing and thawing may degrade the product performance.
4. This product cannot be used in the probe method of fluorescence quantitative PCR.
5. When preparing the reaction solution, use new or non-contaminated tips and tubes to prevent contamination as much as possible.

Usage

The following are examples of conventional PCR reaction systems and conditions, which should be improved and optimized according to the template, primer structure and fragment size.

1. PCR reaction system

reagents	50 μL reaction system	final concentration
2×UltraSYBR Mixture	25 μL	1 ×
Forward Primer, 10 μM	1 μL	0.2 μM [1)]
Reverse Primer, 10 μM	1 μL	0.2 μM [1)]
Template DNA	2 μl [2)]	/
ddH2O	up to 50 μl	/

Attention:

(1) Usually, a primer concentration of 0.2 μM gives better results, and a final concentration of 0.1-1.0 μM can be used as a reference for setting the range.
(2) Usually, the amount of DNA template is 10-100 ng of genomic DNA or 1-10 ng of cDNA as a reference. Since the templates of different species contain different copy numbers of target genes, the templates can be diluted in a gradient to determine the optimal amount of template to be used.
(3) The recommended reaction system is 50 μl, which can be scaled up or down according to the actual experimental needs.

2. PCR reaction program:

Caution! The pre-denaturation reaction of this product must be completed at 95° C for 10 minutes!

It is recommended to use the two-step PCR program shown in the table below, and this program is based on the ABI7500 Fluorescent PCR Instrument as an example. If you cannot get good results due to primers with low Tm values, you can try three-step PCR amplification, see Optimization of Reaction Conditions for more details on the three-step procedure.

move	temp	timing	/
premutability	95° C	10 min [1)]	/
denaturation	95° C	15 s	35-40 cycles
Annealing/Extension[2)]	60° C	1 min	35-40 cycles
Melting curve analysis[3)]	/	/	/
/	95° C	15 s	/
/	60° C	1 min	/
/	95° C	15 s	/
/	60° C	15 s	/

Attention:

(1) The hot-start enzyme used in this product shall be activated under the condition of pre-denaturation 95℃ and 10 min.
(2) Please use 60-64℃ as a reference for setting range of annealing temperature, and increase the annealing temperature when non-specific reaction occurs.

(3) This program is set up with the ABI 7500 Fluorescence PCR Instrument as a reference, and the melting curve analysis should be set up with the program recommended by the Fluorescence PCR Instrument used.

Optimization of reaction conditions

In the optimization of fluorescence quantification reaction conditions, primer concentration, annealing temperature, extension time and other aspects should be considered to improve the reaction specificity and amplification efficiency.

1. An experimental system with high reaction specificity and amplification efficiency should have the following conditions:

(1) High reaction specificity: negative control without non-specific amplification such as primer dimer; no amplification beyond the target fragment.

2) High amplification efficiency: low Ct value; high PCR amplification efficiency, close to the theoretical value of 100%.

2. Methods for optimizing reaction conditions:

1) Primer concentration: Usually, a primer concentration of 0.2 μM can give better results, and a final concentration of 0.1-1.0 μM can be used as a reference for setting the range. If you want to improve the specificity of the reaction, you can reduce the concentration of primer; if you want to improve the amplification efficiency, you can increase the concentration of primer to optimize the reaction system.

2) Annealing temperature: It is recommended to use two-step PCR with annealing temperature of 60℃ for the reaction. If you want to improve the specificity of the reaction, you can increase the annealing temperature, and take 60-64℃ as the reference of the setting range. If you can not get good results due to the use of primers with low Tm values, you can try three-step PCR amplification, and the annealing temperature of the three-step method should be set in the range of 56℃-64℃ as a reference.

(3) Extension time: It is recommended to use two-step PCR with an extension time of 1 min for the reaction. If you want to improve the amplification efficiency, you can try to increase the extension time, or try three-step PCR.

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Three-step fluorescence quantitative PCR (this program is based on the ABI7500 fluorescence quantitative PCR instrument):

move	temp	timing	/
premutability	95° C	10 min [1)]	/
denaturation	95° C	10 s	35-40 cycles
annealing (metallurgy)	56-64° C [2)]	30 s	35-40 cycles

reach	72° C	32 s [3]	35-40 cycles
Melting curve analysis[4]	/	/	/
/	95° C	15 s	/
/	60° C	1 min	/
/	95° C	15 s	/
/	60° C	15 s	/

Attention:

(1) The hot-start enzyme used in this product shall be activated under the condition of pre-denaturation 95℃ and 10 min.

(2) Appropriately reduce the annealing temperature when the desired amplification efficiency cannot be obtained; increase the annealing temperature when a non-specific reaction occurs.

3) If the reaction amplification efficiency needs to be improved, the extension time can be increased appropriately.

(4) This program is set up with the ABI 7500 Fluorescence PCR Instrument as a reference, and the melting curve analysis should be set up with the program recommended for the Fluorescence PCR Instrument used.