# UltraSYBR Mixture (High ROX)

**Project number:** U665701

**Storage condition:** -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

## Product content

| Component | U665701-5ml |
|---|---|
| 2 x UltraSYBR Mixture (High ROX) | 5 x 1ml |
| ddH2O | 5 x 1ml |

## Product Introduction

UltraSYBR Mixture (High ROX) is a premixed system dedicated to dye-based (SYBR Green I) real-time fluorescent quantitative PCR at a concentration of 2×, containing GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I fluorescent dye and Mg2+ and High ROX correction dyes for easy and convenient operation. 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 contained in this product can bind to all double-stranded DNAs, enabling the product to be used for the detection of different target sequences without the need to synthesize specific labeling probes. GoldStar Taq DNA Poly-merase 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 primers and templates or primer dimerization at room temperature, and the enzyme activation must be incubated at 95℃ for 10 minutes. The unique combination of PCR buffer system and hot start enzyme effectively inhibits non-specific PCR amplification and significantly improves PCR amplification efficiency.

The ROX dye contained in this kit can correct the fluorescence signal error generated between the wells of the quantitative PCR instrument. This kit contains a high content of ROX correction dye and is suitable for ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus and other fluorescence and quantitative PCR instruments that require a high ROX signal for calibration. quantitative PCR instruments that require a higher ROX signal for correction.

## Product Features

1. The new high-efficiency hot-start enzyme GoldStar Taq DNA Polymerase is used in this product with a unique PCR buffer system, which significantly improves the amplification efficiency of PCR with high sensitivity and specificity.
2. Suitable for fluorescent quantitative PCR assays, capable of accurately quantifying and detecting target genes.

## matters needing attention

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 and ROX dye. Avoid strong light when storing this product or preparing PCR reaction solution.

3. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may degrade product performance.
4. This product cannot be used for fluorescent quantitative PCR by the probe method.
When preparing reaction solutions, use new or non-contaminated tips and centrifuge tubes to prevent contamination as much as possible.

## Usage

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

1. PCR reaction system

| reagents | 50μl reaction system | final concentration |
|---|---|---|
| 2×UltraSYBR Mixture (High ROX) | 25 μl | 1× |
| Forward Primer, 10μM | 1μl | 0.2μM[1)] |
| Reverse Primer, 10μM | 1μl | 0.2μM[1)] |
| Template DNA | 2μl[2)] | |
| $ddH_2O$ | up to 50μl | |

Note: 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 10-100ng of genomic DNA or 1-10ng of cDNA as a reference. Since the number of copies of the target gene contained in the templates of different species varies, the templates can be subjected to gradient dilution to determine the optimal amount of template to use.
3) The recommended reaction system is 50 μl, which can be scaled up or down according to the actual experimental requirements.

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 shown in the table below to set up the program, and this program is based on the ABI7900 fluorescent quantitative PCR instrument as an example. If you do not get good experimental results due to lower Tm values of primers and other reasons, you can try to carry out three-step PCR amplification, the three-step procedure is detailed in the Optimization of Reaction Conditions.

| Steps | Temperature | time | |
|---|---|---|---|
| Pre denaturation | 95° C | 10min[1)] | |
| denaturation | 95° C | 15s | } 35-40 cycles |
| Annealing/Extension[2)] | 60° C | 1min | |
| Analysis of fusion curve[3)] | | | |
| | 95° C | 15s | |
| | 60° C | 1min | |
| | 95° C | 15s | |
| | 60° C | 15s | |

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

(2) Please use 60-64℃ as the 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 7900 fluorescent quantitative PCR instrument as a reference. For melting curve analysis, please set up the program recommended for the fluorescent quantitative PCR instrument used.

## Optimization of reaction conditions

In the optimization of fluorescence quantification reaction conditions, primer concentration, annealing temperature, and extension time should be considered to improve reaction specificity and amplification efficiency.
1. Experimental systems 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 get better results, and the 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 primer concentration; if you want to improve the amplification efficiency, you can increase the primer concentration, thus optimizing the reaction system.
2) Annealing temperature: It is recommended to use two-step PCR with an annealing temperature of 60° C 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 experimental results due to the use of primers with lower Tm values, etc., you can try to perform three-step PCR amplification, and the annealing temperature of the three-step method, please take the range of 56℃-64℃ as the setting reference.
3) Extension time: it is recommended to use two-step PCR with an extension time of 1min for the reaction. If you want to improve the amplification efficiency, you can try to increase the extension time or try three-step PCR.
Caution! The pre-denaturation reaction of this product must be completed at 95° C for 10 minutes!
Three-step fluorescent quantitative PCR (this program is based on the ABI 7900 fluorescent quantitative PCR instrument):

| move | temp | timing | |
|---|---|---|---|
| premutability | 95° C | 10min[1] ) | |
| denaturation | 95° C | 10s | 35-40 cycles |
| annealing (metallurgy) | 56-64° C[2] ) | 30s | |
| reach | 72° C | 32s[3] ) | |
| Melting curve analysis[4] ) | | | |
| | 95° C | 15s | |
| | 60° C | 1min | |
| | 95° C | 15s | |
| | 60° C | 15s | |

Note: 1) The hot-start enzyme used in this product shall be activated by the enzyme under the condition of pre-denaturation 95℃ and 10min.
(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 7900 Fluorescence Quantitative PCR Instrument as a reference. For melting curve analysis, please set up the program recommended for the fluorescence quantitative PCR instrument used.