UltraSYBR Mixture

Cat. #: W0957-1 (1 mL); W0957-5 (5 mL)

Storage: Store at -20℃ for up to 1 year and avoid freeze-thaw cycles. Stored at 4℃ if frequent used in short time.

Product Components

Cat. No.	Size	2x Ultra SYBR Mixture	ddH_2O
W0957-1	50 μL volume x 40 reactions	1 mL	1 mL
W0957-5	50 μL volume x 200 reactions	1 mL x 5	1 mL x 5

Product Introduction:

The UltraSYBR Mixture is a premixed system for real-time fluorescence quantitative PCR (SYBR Green I), and the concentration is 2×. It contains GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, and Mg2+.

The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription. This product contains the fluorescent dye SYBR Green I which can bind with all double-strand DNA, so that the product can be used for the detection of different target sequences without the need for the synthesis of specific labeled probes. The GoldStar Taq DNA Polymerase in the mixture is a chemically-modified, new efficient hot-start enzyme that does not have polymerase activity at room temperature which prevents non-specific amplification efficiently, and it is activated by incubation at 95°C for 10 minutes.

The combination of a unique PCR buffer system and a hot-start enzyme effectively inhibits non-specific PCR amplification and significantly increases the amplification efficiency of PCR.

This product is suitable for fluorescent qPCR instruments that do not require ROX as a calibration dye, such as Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, and CFX96.

Features:

1. This product uses a new high-performance hot start enzyme (GoldStar Taq DNA Polymerase) and a unique PCR buffer system. It significantly improves the PCR amplification efficiency and has high sensitivity and specificity.

2. This product is suitable for quantitative PCR detection and can accurately quantify and detect the target gene.

Precautions:

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye. Avoid strong light irradiation when storing this product or preparing PCR reaction solution.
3. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may comprise product performance.
4. This product cannot be used for qPCR using probes.
5. When preparing the reaction solution, use new or noncontaminated tips and centrifuge tubes to prevent contamination.

Protocol:

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50 μL PCR reaction	Final Concentration
2x Ultra SYBR Mixture	25 μL	1x
Forward Primer, 10 μM	1 μL	0.2 μM
Reverse Primer, 10 μM	1 μL	0.2 μM
DNA Template	2 μL	-
Super Pfx DNA Polymerase	0.5 μL	1 U / 50 μL
ddH_2O	Up to 50 μL	-

Note:

1) Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 μM.

2) Usually the amount of DNA template is 10-100 ng for genomic DNA or 1-10 ng for cDNA. Template can be gradient diluted to optimize.

3) The recommended reaction volume is 50 μl, and the reaction volume can also be scaled up or down according to actual experimental requirements.

2. PCR reaction program:

Please notice that the initial denaturation step prior to PCR must be at 95℃ for 10 minutes!

It is recommended to use 2-step PCR reaction program. This program uses ABI7500 qPCR machine as an example. If no good result obtained due to the low Tm of the primers, try a 3-step PCR program.

2-step PCR protocol

Procedure	Temperature	Time	
Initial denaturation	**95℃**	**10 min**	
Denaturation	95℃	15 s	35-40 cycles
Annealing / Extension	60℃	1 min.	
Melting curve analysis			
	95℃	15 s	
	60℃	1 min.	
	95℃	15 s	
	60℃	15 s	

Note:

1) The hot-start enzyme used in this product must be pre-denatured at 95°C for 10 minutes to activate the enzyme.

2) The annealing temperature should be between 60-64°C. If there is non-specific reaction, increase the annealing temperature.

3) This program uses ABI7500 qPCR machine as an example. The melting curve analysis should be set according to the procedure recommended by qPCR instrument used.

Optimization of reaction conditions:

When optimizing the qPCR reaction conditions, different aspects such as the concentration of the primer, the annealing temperature, and the extension time should be considered, to improve the reaction specificity and amplification efficiency.

1. The experimental system with high reaction specificity and high amplification efficiency should be as the following conditions:

   1) High specificity: no non-specific amplification such as primer dimers for negative control; No other amplification beyond the target fragment.

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

2. Optimization of reaction conditions:

1) Primer concentration: Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 μM. To increase the specificity of the reaction, decrease the concentration of the primer; To increase the amplification efficiency, increase the concentration of the primer.

2) Annealing temperature: It is recommended to use 2-step PCR, and set the annealing temperature as 60°C. To increase the specificity, increase the annealing temperature, which should be between 60-64°C. If a good result cannot be obtained due to the low Tm of the primers, try a 3-step PCR program. The annealing temperature of the 3-step PCR program should be between 56°C and 64°C.

3) Extension time: It is recommended to use two-step PCR and set the extension time as 1 minute. To increase amplification efficiency, increase the extension time, or try 3-step PCR.

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

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