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UltraSYBR Mixture

Catalog Number: CW0957S (1ml)
CW0957M (5ml)
CW0957L (25ml)

Storage Condition: -20°C; if used frequently, store at 2-8°C to avoid repeated freezing and thawing.

Kit Components:

Component	CW0957S (1 ml)	CW0957M (5 ml)	CW0957L (25 ml)
2XUltraSYBR Mixture	1 ml	5x1 ml	5x (5x1 ml)
ddH_2O	1 ml	5x1 ml	5x (5x1 ml)

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 Mg^{2+}. 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. This product 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 non-contaminated 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 ul	Final Conc.
2xUltraSYBR Mixture	25 ul	1x
Forward Primer, 10 uM	1 ul	0.2 uM
Reverse Primer, 10 uM	1 ul	0.2 uM
DNA template	2 ul	
Super Pfx DNA Polymerase	0.5 ul	1 U/50 ul
ddH_2O	Up to 50 ul	

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:

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

It is recommended to use two-step PCR reaction program. This program uses ABI7500 qPCR machine as an example. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program.

Procedure	Temperature	Time	
Pre-denaturation	95°C	10 min	
Denaturation	95°C	15 s	35-40 cycles
Annealing/Extension	60°C	1 min	
Melting curve analysis			
	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	60°C	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. Methods of 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 two-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 three-step PCR program. The annealing temperature of the three-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 three-step PCR.

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

Three-step QPCR method

(this program uses ABI7500 QPCR machine as an example)

Procedure	Temperature	Time	
Pre-denaturation	95°C	10 min	
Denaturation	95°C	10 s	35-40 cycles
Annealing	56-64°C	30 s	
Extension	72°C	32 s	
Melting curve analysis			
	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	60°C	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) If good amplification efficiency cannot be achieved, lower the annealing temperature appropriately. If there is non-specific reaction, increase the annealing temperature.

3) To increase amplification efficiency, increase extension time appropriately.

4) 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.