Tinzyme Co., Limited

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Universal Super SYBR Master Mix

Product Number: PCM60

Shipping and Storage

Storage at -20 ± 5 ℃.

Components

Component	PCM60S	PCM60M	PCM60L
2×Universal Super SYBR Master Mix	1 mL	5×1 mL	40×1 mL
ddH_2O	1 mL	5×1 mL	40×1 mL

Description

Universal Super SYBR Master Mix is a specialized premix for real-time fluorescence quantitative qPCR reactions using the dye method (SYBR Green I).The core component FastStar DNA Polymerase is an antibody based hot start DNA polymerase that can be restored by heating at 95℃ for 5 seconds. It has many advantages such as strong specificity and high detection sensitivity, and is paired with an optimal buffer optimized for qPCR. The reaction solution concentration is 2×。The unique qPCR buffer system of this product, combined with hot start enzymes, effectively inhibits the production of non-specific products and significantly improves the amplification efficiency of qPCR. It is very suitable for qPCR reactions with high specificity, sensitivity, and AT, GC models.

This product is also suitable for qPCR rapid reaction programs, small volume reactions, and can achieve rapid and accurate detection and quantification of target genes without affecting specificity and high sensitivity.Good standard curves can be obtained within a wide quantitative range, with accurate, reproducible, and reliable quantification of target genes. The ROX Reference Dye contained in this product is suitable for most qPCR instruments. This product is not easy to generate bubbles when shaking, and the reaction only requires the addition of primers and templates, which is simple and convenient to operate.

Application

This reagent is used for quantitative amplification of DNA samples and can amplify DNA from most species. Sample types can be genomic DNA, cDNA, plasmid DNA λ DNA, etc.

Note

1. Before use, gently mix upside down and centrifuge briefly before use.
2. This product should avoid repeated freeze-thaw as much as possible, and should not be freeze-thawed more than 10 times to avoid a decrease in enzyme activity.
3. Due to its extremely high detection sensitivity, this product is easily contaminated by aerosols in the air. Therefore, when preparing the reaction system, please use a super clean workbench. During the preparation process, please use a sterilization gun head and reaction tube. If conditions permit, it is recommended to use a dedicated pipette gun and a gun head with a filter element in laboratories.
4. Due to the presence of fluorescent dye SYBR Green I in this product, it is important to avoid strong light exposure when preparing the reaction system.

Protocol

The following examples are the conventional qPCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. qPCR reaction system

Reagent	20μL reaction system	Final Concentration

2×Universal Super SYBR Master Mix	10 μL	1×
Forward Primer, 10 μM	0.4μL	0.2μM[1)]
Reverse Primer, 10 μM	0.4μL	0.2μM[1)]
Template DNA[2)]	X μL	
ddH_2O	Up to 20 μL	

The amount of each component in the reaction system can be adjusted according to the following principles:

1)Usually, a primer concentration of 0.2μM at the final concentration can yield good results, and can be used as a reference for setting the range from 0.1μM to 1.0μM at the final concentration.In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2)Usually, the amount of DNA templates is based on 10-100ng genomic DNA or 1-10 ng cDNA. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal template usage.If the template type is undiluted cDNA stock solution, the volume used should not exceed 1/10 of the total volume of qPCR reaction.

2. qPCR reaction program

Step	Temperature	Time	cycles
Pre denaturation[1)]	95℃	5-30s	1
Denaturation[2)]	95℃	5-15s	40-45
Annealing/Extension[3)]	60℃	10-30s*	
	95℃	15s	
Melting curve[4)]	60℃	1min*	
	95℃	1s	

Note:1)This pre denaturation condition is suitable for the vast majority of amplification reactions, with a standard program of 30 seconds and a fast program of up to 5s.If the template structure is complex, the pre denaturation time can be extended to 3 minutes to improve the pre denaturation effect.

2)Standard program selection 15s; Quick program with a minimum optional 5s

3)Standard program selection 30s; Quick program: For amplicons within 200bp, the minimum extension time can be set to 10s; When it exceeds 200 bp, the recommended extension time is 30s

4)Please set the fusion curve analysis using the recommended program for the fluorescence quantitative PCR instrument used. This program is based on the ABI-Q5 fluorescence quantitative PCR instrument as a reference setting.

***Set signal acquisition at the annotation point**