Tinzyme Co., Limited

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2×Universal Fast SYBR Master Mix

Product Number: PCM88

Shipping and Storage

Stored at -20℃, transported in ice bags, Mix can be stably stored for 6 months under dark conditions at 2~8℃ after thawing.

Components

Component	PCM88	PCM88	PCM88
2×Universal Fast SYBR Master Mix	1ml	5×1ml	40×1ml
ddH_2O	1ml	5×1ml	40×1ml

Note: contains dNTP、Mg^{2+}、FastStar DNA Polymerase、ROX Reference Dye etc.

Description

The 2× Universal Fast 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 high specificity and sensitivity qPCR reactions.

This product is also suitable for qPCR rapid reaction program, 10 μ L small volume reaction 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.

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, please gently mix it upside down and avoid foaming as much as possible. After briefly centrifuging, use it. During the sample addition process, blow gently. If Mix bubbles due to improper operation, it needs to be centrifuged again before use.
2. Try to avoid repeated freeze-thaw cycles of this product, with freeze-thaw cycles (≤ 10) to avoid a decrease in enzyme activity. It is recommended to use small portions separately.
3. Due to the presence of fluorescent dye SYBR Green I in this product, it is necessary to store it away from light. When preparing the reaction system, it is advisable to avoid strong light exposure as much as possible.
4. 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.
5. This product contains ROX Reference Dye that is suitable for most models.

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. PCR reaction system

Reagent	20μL	Final Conc.
2×Universal Fast SYBR Master Mix	10μl	1×
Forward Primer，10μM	0.4μl	0.2μM[1)]
Reverse Primer，10μM	0.4μl	0.2μM[1)]
Template DNA[2)]	Xμl	
ddH_2O	up to 20μl	

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

1)Typically, the final concentration of the primer is 0.2μM can obtain good results, which can be achieved at a final concentration of 0.1-1.0μM serves as a reference for setting the range. 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 1-10ng 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. PCR reaction program:

Step	Temperature	Time	cycles
Pre denaturation	95℃	5-30 s	1
Denaturation	95℃	3-10 s	40-45
Annealing/Extension	60℃	10-30 s	
	95℃	15 s	
Final Extension	60℃	1 min	
	95℃	1 s	

Note:1)This pre denaturation condition is suitable for the vast majority of amplification reactions. 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 for 30 seconds; Quick program can be selected for a minimum of 5 seconds. For amplicons within 200bp, the minimum extension time can be set to 3-10 seconds; When it exceeds 200bp, the recommended extension time is 30 seconds.

3)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 58-62℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56-64℃ as a reference.

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