Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Fast SYBR Mixture

Product Number: PCM55

Shipping and Storage

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

Components

Component	PCM55 5ml	PCM55L 5ml	PCM55H 5ml
2×FastSYBR Mixture	5×1mL	5×1mL	5×1mL
50×Low ROX	-	200µl	-
50×High ROX	-	-	200µl
ddH_2O	5×1mL	5×1mL	5×1mL

Description

The Fast SYBR Mixture is a premixed system for real-time fluorescence quantitative PCR (SYBR Green I), and the concentration is 2×. It contains Fast 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 Fast Taq DNA Polymerase in the mixture is a chemically-modified, new efficient enzyme which reduce non-specific amplification efficiently, and it is activated by incubation at 95°C for 20 seconds, which will efficiently reduce the total reaction time.

This product is suitable for fluorescent qPCR instruments with no ROX.

No ROX as a calibration dye: Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, and CFX96.

Need Low ROX (30-50mM) as a calibration dye: ABI Prism7500/7500 Fast, QuantStudio® 3System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System,ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000

Need High ROX (300-500mM) as a calibration dye: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus.

Features

This product uses a new high-performance hot start enzyme (Golden Star Taq DNA Polymerase) and a unique PCR buffer system. This product significantly improves the PCR amplification efficiency and has high sensitivity and specificity. This product is suitable for quantitative PCR detection and can accurately quantify and detect the target gene.

Note

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	50μl	Final Conc.
2×Fast SYBR Mixture	25μl	1×
Forward Primer, 10 μM	1μl	0.2μM [1)]
Reverse Primer, 10 μM	1μl	0.2μM [1)]
Template DNA	2μl[2)]	
50×Low ROX or High ROX(optional)[3)]	1μl	1×
ddH_2O	up to 50μl	

Note:. 1) Usually, a primer concentration of 0.2μM can yield good results, and can be used as a reference for setting the range from 0.1 to 1.0μM. 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.

3) The excitation optical systems of different instruments vary, and 50×Low ROX or 50×High ROX can be added depending on the instrument used for fluorescence quantification.

2. PCR reaction condition:

Procedure	Temperature	Time	
Pre-denaturation	95℃	20sec	
Denaturation	95℃	3sec	} 35-40cycles
Annealing/Extension	60℃	30sec	
Melting curve analysis			
	95℃	15sec	
	60℃	1min	
	95℃	15sec	
	60℃	15sec	

Note: 1)The enzyme used in this product must be activated under pre denaturation conditions of 95℃ and 20 seconds. Under this condition, most templates can perform well in de chaining. For templates with high GC content and complex secondary structures, the pre denaturation time can be extended to 1 minute to fully unwind the starting template. If the high-temperature treatment time is too long, it will affect the enzyme activity. The optimal pre denaturation time can be determined based on the template situation.

2)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 60-64℃ 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 method can be attempted. The annealing temperature should be set within the range of 56℃ -64℃ as a reference.

3)Please set the fusion curve analysis using the recommended program for the fluorescence quantitative PCR instrument used. This program is based on the ABI 7500 fluorescence quantitative PCR instrument as a reference setting.