Tinzyme Co., Limited

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

2×Quick Probe Mixture

Product Number: PCM27

Shipping and Storage

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

Components

Component	PCM27 5ml	PCM28 5ml	PCM29 5ml
2×Quick Probe 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 2×Quick Probe Mixture a premixed system specifically designed for real-time fluorescence quantitative PCR using probe methods (TaqMan, Molecular Beacon, etc.), and the concentration is 2×. It contains Quick Taq DNA Polymerase、PCR Buffer、dNTPs、Mg^{2+} etc. The operation is simple and convenient.Mainly used for detecting genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.This product contains Fast Taq DNA Polymerase, which can effectively reduce non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. The activation of the enzyme only requires incubation at 95℃ for 30 seconds.The entire PCR reaction process can save about 40 minutes compared to ordinary reactions, greatly shortening the reaction time of PCR. The combination of a unique PCR buffer system and rapid hot start enzymes effectively inhibits the production of non-specific products and significantly improves the amplification efficiency of PCR. The fluorescence signal is stronger, the sensitivity is higher, and the linear range is wider. This product has a wide range of applications and can be used for both regular and rapid quantitative PCR programs.

ROX dye is used to correct the fluorescence signal errors generated from hole to hole in quantitative PCR instrument. It is generally used in ABI, Stratagene and other companies' Real Time PCR amplifiers. The excitation optical system varies from instrument to instrument, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument.

Instruments without ROX correction:Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX correction:ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, etc.

Instruments requiring High ROX calibration:ABI Prism7000/7300/770/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Notes

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored for a long time at -20℃, away from light. If frequent use is required in the short term, it can be stored at 2-8℃.

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×Quick Probe Mixture	25μl	1×
Forward Primer,10μM	1μl	0.2μM [1)]
Reverse Primer,10μM	1μl	0.2μM [1)]
Probe，10μM	1μl	0.2μM [2)]
Template DNA	2μl[3)]	
50×Low ROX or High ROX(optional)[4)]	1μl	1×
ddH_2O	up to 50μl	

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

2)The final concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)The amount of DNA templates is usually based on 10-100ng genomic DNA or 1-10ng cDNA as a reference. 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.

4)The excitation optical systems of different instruments vary,choose to add according to the instrument using fluorescence quantification 50× Low ROX or 50×High ROX

2. PCR reaction program:

It is recommended to use two-step PCR reaction program. This program uses ABI7500 qPCR machine as an example.

Step	Temperature	Time	
Pre-denaturation	95℃	30s[1)]	
Denaturation	95℃	5s	35-40cycles
Annealing/Extension	60℃	30s	

Note:1)The enzyme used in this product must be activated under pre denaturation conditions of 95℃ and 30 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 1-4 minutes to fully unwind the starting template.

2)It is recommended to use a two-step PCR reaction program. 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.