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Universal Probe qPCR Mix

Product Number: PCM121

Shipping and Storage

For long-term storage, please store in the dark at -20℃. Mix can be stably stored for one month at 4 ℃ after melting, avoiding repeated freezing and thawing as much as possible.

Components

Component	Specifications
Universal Probe qPCR Mix	5×1mL

Description

Universal Probe qPCR Mix is a real-time quantitative PCR 2 x premix developed based on a unique two-component hot start Taq polymerase. This product contains all fluorescent quantitative PCR components except primers and sample DNA, which can reduce operating steps, shorten sample addition time, and lower the risk of contamination. It is suitable for TaqMan probe detection using cDNA or DNA as samples.

This product contains unique calibration dyes and is compatible with a range of qPCR devices, including instruments that require ROX calibration. No additional dyes need to be added to calibrate the instruments during experimental operations.

Protocol

1. Precautions for use

1.1. Due to the pre mixed dye in Mix, its storage or reaction system preparation process should avoid exposure to strong light.

1.2. Before use, gently mix the Mix by flipping it up and down. Do not vortex or shake the mixture to avoid generating too many bubbles.

1.3. Mix contains Universal calibration dyes, suitable for all models, without the need for additional dyes.

2. Suggested qPCR reaction system

Reagent	Usage	Final concentration
Universal Probe qPCR Mix	10μL	1×
Positive Primer (10μM) a	0.4μL	0.2μM
Reverse Primer (10μM) a	0.4μL	0.2 μM
TaqMan probe (5μM) b	1μL	0.25μM
DNA template c	xμL	10~200 ng/20μL
Nuclease-Free Water	To 20μL	

2.1. The recommended final concentration of primers is 0.2μM, and if the effect is not satisfactory, it can be adjusted between 0.1~1μM; Please set the primer length to 18-25bp and the GC content to 40% -60%. The optimal amplification target fragment for the best efficiency is generally 80-200bp. When designing, it is advisable to avoid complex structures such as hairpin structures and dimers, and to span the intron region as much as possible;

2.2. The recommended final concentration of the probe is 0.25μM, and if the effect is not satisfactory, it can be adjusted between 0.1~1μM;

2.3. The amount of template added should not exceed 10% of the total reaction system, and the recommended sample amount is 1-2μL. Different types of DNA templates contain different numbers of target gene copies, and gradient dilution may be necessary to determine the optimal amount of DNA template to be added.

3. qPCR reaction program (can be adjusted according to the model)

Two-step method

Step	Temperature	Time	Number of cycles
Pre denaturation	95℃	5 min	
Denaturation	95℃	10s	40 Cycles
Annealing&Extension	60℃	30s	

Note

1. Before use, please invert and gently mix up and down to avoid foaming, and centrifuge briefly before use.
2. Avoid repeated freeze-thaw mixing and try to use it up within 3 months after opening.

Frequently asked questions

Problem description	Possible reasons	Solution
Chaotic or missing amplification curves	Incorrect instrument settings	Adjust the settings according to the instrument manual
	Improper concentration of primers or templates	Adjust primer and template concentrations
	Improper PCR reaction conditions	Reduce annealing temperature, extend extension time, etc. For target fragments with high GC content, the denaturation time can be appropriately extended
	Primers or templates have advanced structures	Redesign primers
	Poor purity of the sample	Further purify the sample
Poor repeatability of quantitative values	Incorrect instrument settings	Adjust the settings according to the instrument manual
	Poor purity of the sample	Further purify the sample
	Improper primer concentration	Attempt to increase the primer concentration appropriately
	Improper PCR reaction conditions	Attempt to lower annealing temperature, extend extension time, etc
	Improper primer design	Redesign primers to reduce the high-level structure of target fragments
	Experimental operation error	Strictly follow the operating procedures to ensure accurate volume of each component in the reaction system
Signal appears in blank control	Pollution occurs	Firstly, replace the blank control with water; If the same situation occurs again, continue to replace primers, gun heads, PCR tubes, or enable a new Mix