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Universal SYBR qPCR Mix

Product Number: PCM117

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 as much as possible.

Components

Component	Specifications
Universal SYBR qPCR Mix	5×1mL

Description

Universal SYBR qPCR Mix is SYBR ® Green I chimeric dye method specific qPCR reagent is a 2×premix containing all qPCR components except primers and DNA samples, which can reduce operating steps, shorten sample addition time, and lower the risk of contamination. Its core component is antibody modified hot start Taq DNA polymerase, combined with carefully optimized buffer system and PCR reaction promoting factors, making the product highly specific and efficient, effectively inhibiting non-specific amplification, and accurately determining the amount of templates in a wide concentration range to obtain stable and reliable qPCR results.

The premix contains unique calibration dyes that are compatible with a range of qPCR devices, including instruments that require ROX calibration. No additional dyes need to be added during the experimental operation to calibrate the instruments.

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 SYBR qPCR Mix	10μL	1x
Positive Primer (10μM) [a]	0.4μL	0.2μM
Reverse Primer (10μM) [a]	0.4μL	0.2μM
DNA template [b]	XμL	10~200ng/20μL
Nuclease-Free Water	To 20μL	

2.1. The recommended final concentration of primers is usually 0.2μM, and adjustments can be made within the range of 0.1~1μM when the reaction effect is poor;

2.2. The recommended template dosage is 1-2μL. If the template type is undiluted cDNA stock solution, the template dosage should not exceed 10% of the total reaction system. 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.

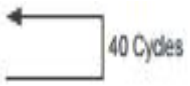
3. qPCR reaction program (can be adjusted according to the model)

3.1. Two-step method

Step	Temperature	Time	
Pre denaturation	95℃	30s	
Denaturation	95℃	10s	40 Cycles
Annealing&Extension [a]	60℃	30s	

Melting curve [b]	Use the default acquisition program of the instrument	

3.2. Three-step approach

Step	Temperature	Time	
Pre denaturation	95℃	30s	
Denaturation	95℃	10s	
Annealing [a]	55-60℃	10s	
Extension [a]	72℃	30s	
Melting curve [b]	Use the default acquisition program of the instrument		

3.3. Set the annealing and extension (annealing) temperature based on the Tm value of the primer; If the amplified fragment is within 200bp, the annealing and extension time can be set to 15 seconds. In addition, the annealing and extension time settings need to be adjusted according to the minimum data acquisition time required by the qPCR instrument you are using;

3.4. The melting curve acquisition program of different qPCR instruments varies, and the default melting curve acquisition program of the instrument can generally be used.

Experimental optimization

If the default reaction conditions have poor reaction performance, optimization of the reaction conditions is needed, which can be carried out from two aspects: primer concentration and amplification program:

1. Primer concentration adjustment: When the final primer concentration varies between 0.1~1.0μM, the lower the primer concentration, the higher the amplification specificity, but the amplification efficiency will decrease.
2. Amplification program optimization: To improve amplification specificity, a two-step program or increasing annealing temperature can be used; To improve amplification efficiency, a three-step procedure or extended extension time can be used.

Primer design principles

1. It is recommended to control the length of the amplified product within 80-200bp;
2. Primer length is 18-25bp;
3. The difference in Tm values between forward and reverse primers should not exceed 1℃, and the Tm value should be controlled between 58-62℃;
4. The GC content of primers should be controlled between 40% and 60%;
5. The overall distribution of primers A, G, C, and T should be as uniform as possible, avoiding continuous structures of T/C or A/G (especially the 3 'end);
6. The last base at the 3 'end of primer is preferably G or C;
7. Avoid complementary sequences within primers or between two primers;
8. Use NCBI BLAST function to search and confirm the specificity of primers.

Frequently asked questions

Problem description	Possible reasons	Solution
The amplification curve is not smooth	The fluorescence signal is too weak, and after system correction, it generates	Ensure that the pre mixed dyes in the Mix are not degraded; Replace qPCR specialized consumables with better fluorescence signal collection
Amplification curve breakage or decline	The template concentration is high, and the endpoint value of the baseline is greater than the Ct value	Reduce the baseline endpoint (Ct value -4) and reanalyze the data
Sudden drop in amplification curve of individual wells	There are bubbles left inside the reaction tube	Ensure that the Mix is completely dissolved and do not vortex or shake to mix evenly After adding the sample, gently centrifuge to remove bubbles

		Extend the pre denaturation time to 10 minutes to remove bubbles
The reaction ends without any amplification curve appearing	Insufficient number of reaction cycles	Set the number of loops to 40, but more loops will increase the background signal too much
	Fluorescence signal acquisition steps not set or set incorrectly	The two-step amplification program generally sets the signal acquisition in the annealing&extension stage, while the three-step amplification program should set the signal acquisition in the 72℃ extension stage
	Primers may degrade	Primers that have not been used for a long time should be tested for integrity by PAGE electrophoresis to rule out the possibility of degradation
	Template concentration too low	Reduce the dilution factor of the template and repeat the experiment, starting from the highest concentration when the sample concentration is unknown
	Template degradation	Prepare the template again and repeat the experiment
Cq value appears too late	Low amplification efficiency	Increase primer concentration, try three-step amplification program, or redesign primers
	Template concentration too low	Reduce the dilution factor of the template and repeat the experiment, starting from the highest concentration when the sample concentration is unknown
	Template degradation	Prepare the template again and repeat the experiment
	The amplification product is too long	Control the length of the amplified product within 80-200bp
	PCR inhibitors exist in the system	Usually, it is carried in as a template, increasing the dilution factor of the template or preparing a high-purity template for repeated experiments
Signal appears in blank control	Pollution of reaction system	Firstly, replace the blank control with water. If the same situation occurs again, continue to replace primers, probes, PCR tubes, or enable a new Mix The reaction system is prepared in an ultra clean workbench to reduce aerosol pollution
	Non specific amplification such as primer dimers appears	It is normal for the blank control to show an increase in yield after 35 cycles, and it should be analyzed in conjunction with the melting curve Redesign primers, adjust primer concentration or optimize PCR reaction program
The melting curve shows multiple peaks	Poor primer design	Redesign new primers based on primer design principles
	Primer concentration too high	Reduce primer concentration appropriately
	CDNA template contains genomic contamination	The extracted RNA solution is digested using DNA enzymes, such as dsDNase, to remove genomic contamination, or to design cross intron primers
Poor repeatability of the experiment	Large sample addition error	Using precise pipettes and high-quality suction tips to accurately transfer high dilution templates, adding large volume templates to reduce sample errors Amplify qPCR reaction volume
	Template concentration too low	Reduce template dilution factor and repeat experiments

	Temperature deviation at different positions of qPCR instrument	Regularly calibrate qPCR instrument