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Universal Visual SYBR qPCR Master Mix

Product Number: PCM18

Shipping and Storage

For long-term storage, please store in the dark at -20°C. After thawing, it can be stably stored for one month at 4°C in the dark, avoiding repeated freezing and thawing as much as possible.

Component

Component	PCM18	PCM18
Universal Visual SYBR qPCR Master Mix	4×1.25mL	20×1.25mL
10× Dilution Buffer	1mL	5×1mL

Description

Universal Visual SYBR qPCR Master Mix is a specialized qPCR reagent for SYBR Green I chimeric dye method. It is a 2 × premix containing all qPCR components except primers and DNA samples, which can reduce operation 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 promoter, which has strong specificity and high amplification efficiency, and can effectively inhibit non-specific amplification. It can accurately quantify templates with a wide concentration range and obtain stable and reliable qPCR results. This product already contains universal calibration dyes and is compatible with the vast majority of qPCR devices, without the need for additional dyes to calibrate the instrument.

This product can utilize the color changing effect generated by mixing different dyes to track the pipetting process, thereby significantly reducing pipetting errors. The Universal Visual SYBR qPCR Master Mix contains blue dye, while the 10 × Dilution Buffer contains yellow dye. When a template diluted with Dilution Buffer (yellow) is added to the Universal Visual SYBR qPCR Master Mix (blue), a color change effect from blue to green occurs, allowing for accurate determination of whether the template has been added based on the liquid color.

Protocol

1. Template dilution

During use, if pipetting tracking is required, select the appropriate method according to the table below to add Dilution Buffer to the template in advance, and then perform qPCR detection; If pipetting tracking is not required, Dilution Buffer should not be used.

DNA template status	Usage of 10 × Dilution Buffer	Dilution Buffer concentration in the template
Solid	Dilute 10 × Dilution Buffer with ddH_2O to 1 ×, and dissolve DNA in 1 × Dilution Buffer	1 ×
Solution	If necessary, first dilute the template to the target concentration using ddH2O, and then add 1μL of 10 × Dilution Buffer to every 9μL of template	1 ×

Note: Improper use of Dilution Buffer may affect qPCR results.

2. Suggested qPCR reaction system

Reagent	Usage	Final concentration
Universal Visual SYBR qPCR Master Mix	10μL	1 ×
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	

a. The recommended final concentration of primers is usually 0.2μM, which can be adjusted within the range of 0.1~1μM;

b. If using Dilution Buffer for sample tracking, the template volume should not exceed 2-5μL/20μL reaction. If the template dosage is less than 2μL/20μL reaction, it will cause color fading and affect the tracking effect; If the template dosage is higher than 5μL/20μL reaction, the components in the Dilution Buffer may interfere with the qPCR reaction. No

The number of target gene copies contained in the same type of DNA template varies, and gradient dilution may be necessary to determine the optimal amount of DNA template to be added.

Note: When the template type is undiluted cDNA stock solution, regardless of whether it contains 1 × Dilution Buffer, the volume used should not exceed 1/10 of the total volume of qPCR reaction, that is, 2μL/20μL reaction.

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

3.1. Two-step method

Step	Temperature	Time	cycles
Pre denaturation	95°C	30s	
Denaturation	95°C	10s	40 cycles
Annealing&Extension [a]	60°C	30s	
Melting curve	Use the default acquisition program of the instrument		

3.2. Three-step approach

Step	Temperature	Time	cycles
Pre denaturation	95°C	30s	
Denaturation	95°C	10s	40 cycles
Annealing	55-65°C	10s	
Extend	72°C	30s	
Melting curve	Use the default acquisition program of the instrument		

a. 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.

4. Experimental optimization

4.1. Primer concentration adjustment

When the final concentration of primers varies between 0.1~1.0μM, the lower the primer concentration, the higher the amplification specificity, but the amplification efficiency will decrease.

4.2. Amplification program optimization

To improve amplification specificity, a two-step procedure or increasing annealing temperature can be used; To improve amplification efficiency, a three-step procedure or extended extension time can be used.

5. Primer design principles

5.1. It is recommended to control the length of the amplified product within 80-200bp;

5.2. Primer length is 18-25bp;

5.3. The difference between the two Tm values should not exceed 1°C, and the Tm value should be controlled between 58-62°C;

5.4. The GC content of primers should be controlled between 40% and 60%;

5.5. 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), and the last base at the 3' end should preferably be G or C;

5.6. Avoid complementary sequences within primers or between two primers;

5.7. Using NCBI BLAST function to search and confirm the specificity of primers.

FAQ

1. Question: The amplification curve is not smooth

1.1. Possible reasons: The fluorescence signal is too weak, and after system correction, it generates

Answer: Ensure that the pre mixed dyes in the Mix are not degraded; Replace qPCR specialized consumables with better fluorescence signal collection.

2. **Question: Amplification curve breakage or decline**

2.1. Possible reasons: The template concentration is high, and the endpoint value of the baseline is greater than the Ct value.

Answer: Reduce the baseline endpoint (Ct value -4) and reanalyze the data.

3. **Question: Sudden drop in amplification curve of individual wells**

3.1. Possible reasons: There are bubbles left inside the reaction tube.

Answer: Ensure that the Mix is completely dissolved and do not vortex or shake to mix evenly; After adding the sample, gently centrifuge to remove any bubbles; Extend the pre denaturation time to 10 minutes to remove bubbles.

4. **Question: The reaction ends without any amplification curve appearing**

4.1. Possible reasons: Insufficient number of reaction cycles

Answer: Set the number of loops to 40, but more loops will increase the background signal too much.

4.2. Possible reasons: Fluorescence signal acquisition steps not set or set incorrectly

Answer: 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°C extension stage.

4.3. Possible reasons: Primers may degrade

Answer: 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.

4.4. Possible reasons: Template concentration too low

Answer: Reduce the dilution factor of the template and repeat the experiment, starting from the highest concentration when the sample concentration is unknown.

4.5. Possible reasons: Template degradation

Answer: Prepare the template again and repeat the experiment.

5. **Question: Ct value appears too late**

5.1. Possible reasons: Pollution of reaction system

Answer: 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.

5.2. Possible reasons: Non specific amplification such as primer dimers appears

Answer: It is normal for the blank control to show an increase in yield after 35 cycles and should be analyzed in conjunction with the melting curve; Redesign primers, adjust primer concentration or optimize PCR reaction program.

6. **The melting curve shows multiple peaks**

6.1. Possible reasons: Poor primer design

Answer: Redesign new primers based on primer design principles.

6.2. Possible reasons: Primer concentration too high

Answer: Reduce primer concentration appropriately.

6.3. Possible reasons: CDNA template contains genomic contamination

Answer: The extracted RNA solution is digested using DNA enzymes, such as dsDNase, to remove genomic contamination, or to design cross intron primers.

7. **Poor repeatability of the experiment**

7.1. Possible reasons: Large sample addition error

Answer: Use precise pipettes and high-quality suction tips for accurate pipetting; Dilute the template at a high magnification and add a large volume template to reduce sampling errors; Amplify qPCR reaction volume.

7.2. Possible reasons: Template concentration too low

Answer: Reduce template dilution factor and repeat experiments.

7.3. Possible reasons: Temperature deviation at different positions of qPCR instrument

Answer: Regularly calibrate qPCR instrument.