F488 SYBR qPCR Mix (Universal)

REF: EG23111L

Storage Condition

Store at -30~-15℃ and protect from light. Transport at ≤0℃ .

Components

Component	EG23111L
F488 SYBR qPCR Mix (Universal)	25×1 ml

Description

F488 SYBR qPCR Mix (Universal) is a special premix for qPCR detection using SYBR Green I chimeric fluorescence method. The Mix contains all components for qPCR, except DNA templates and primers. The core component is the updated hot-start Taq DNA polymerase with better specificity. With the optimized buffer, the mix is able to obtain consistent and reliable qPCR results in a broad template concentration range.

This product contains universial reference dye compatible with most of qPCR devices, and do not require additional ROX dyes before reaction.

Protocol

1. Notes

① Avoid strong light exposure during usage and storage to protect the pre-mixed dye

② Mix gentle and thoroughly by turning the tube up and down before use. Do not vortex to generate excessive bubbles;

③ This mixture contains universal corrective dyes for all qPCR instrucments.

2. Prepare the following mixture in a qPCR tube

Reagent	Amount	Final concentration
F488 SYBR qPCR Mix	10 μl	1×
Forward primer (10 μM)[a]	0.4 μl	0.2 μM
Reverse primer (10 μM)[a]	0.4 μl	0.2 μM
DNA template[b]	X μl	10~200 ng/20 μl
Nuclease-Free Water	To 20 μl	

a. Commonly used primer concentration: 0.2 μM final. Adjust between 0.1~1 μM;

b. Suggested template volume: 1~2 μl. Do not exceed 10% of the total volume if the template is undiluted cDNA. As the copy number of target gene in different DNA templates varies, gradient dilution can be performed if necessary to determine the optimal amount of DNA template.

3. qPCR program（Adjust according to the instrument used）

2-step PCR

Step	Temperature	Time	
Pre-denaturation	95 ℃	30~60 s	
Denaturation	95 ℃	10 s	40 Cycles
Annealing & Extension[a]	60 ℃	30 s	
Melting curve[b]	Set according to the instrument used		

3-step PCR

Step	Temperature	Time	
Pre-denaturation	95 ℃	30~60 s	
Denaturation	95 ℃	10 s	40 Cycles
Annealing[a]	55~65 ℃	10 s	
Extension[a]	72 ℃	30 s	
Melting curve[b]	Set according to the instrument used		

a. Set Annealing or Annealing & Extension temperatures according to the Tm of primers. Set Extension or Annealing & Extension time at 15 s for <200 bp amplifications. Please also consider the minimum data acquisition time of the instrument used for the time setting.

b. Default programs of instrument are usually used.

4. Optimizations

When ideal results are not obtained using the default conditions, optimizations are necessary:

① Primer concentration adjustment

When final concentration of primers is in the range of 0.1~1.0 μM, the lower concentration leads to the higher the amplification specificity, but the amplification efficiency will decrease.

② Primer design principles

If higher amplification specificity is desired, use the 2-step PCR or increase the annealing temperature. If higher amplification efficiency is desired, use the 3-step PCR or increase the extension time.

5. Primer design principles

① Suggested amplification length: 80~200 bp;

② Primer length: 18~25 bp;

③ Difference of the Tm between two primers should less than 1 ℃ . Suggested Tm range: 58~62℃ ;

④ Primer GC content: 40%~60%;

⑤ Keep an uniform distribution of A, G, C, and T in the primers; Avoid repeated T/C or A/G (especially near the 3' end);

⑥ The 3' end of a primer is better to be a C or a G;

⑦ Avoid complementary sequences in a primer or between two primers;

⑧ Confirm the specificity of primer with NCBI primer-BLAST.

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FAQ & Troubleshooting

Problem	Possible Reason	Solution
Unsmoothed amplification curve	Weak fluorescence signal. Curve generated after system correction	Make sure the pre-mixed dye is not degraded. Or use qPCR consumables with better translucency.
Broken or decreasing amplification curve	The concentration of template is too high. The end value of the base line is higher than Ct.	Lower the base line end (Ct - 4) and re-analyze data.
Sharp decrease of individual amplification curve	Bubbles in the reaction tube.	Completely thaw the mixture. Do not vortex. Tap or centrifuge to remove the bubbles after setting up the reaction mixture. Pre-denature for 10 min to remove bubbles.
No amplification curve	Not enough cycle	Set cycle numbers to 40. Please note background noise will increase with excessive cycles.
	None or improper setting of the fluorescence signal acquisition step.	Set signal acquisition at the Annealing & Extension step for the 2-step cycle or at the Extension step for the 3-step cycle.
	Primer degradation.	Check the quality of primers stored for a long time by PAGE.
	Low template concentration.	Reduce the dilution ratio of the template. For templates with unknown concentrations, start with the highest possible concentration.
	Template degradation.G	Prepare fresh template.
Late Ct.	Low amplification efficiency.	Increase primer concentration. Or use the 3-step cycle. Or re-design primer.
	Low template concentration.	Reduce the dilution ratio of the template. For templates with unknown concentrations, start with the highest possible.
	Template degradation.	Prepare fresh template.
	Long amplification product	Control the amplification length to be 80~200 bp.
	Presence of PCR inhibitor.	PCR inhibitors usually come from template. Increase the dilution ratio of the template or prepare purer template
Signal from blank control	Contamination.	Change water, primers, pipette tips, and PCR tubes one by one to find and eliminate the contaminant source. Open a fresh tube of Mix if necessary.Set up the reaction mixture in a clean bench to avoid aerosol contaminations
	Nonspecific amplifications, e.g. primer dimers.	Signals from blank after 35 cycles are normal. Analyze with the melting curve. Re-design primer. Adjust primer concentrations or PCR cycling conditions.
Multiple peaks of the melting curve	Poor primer design.	Re-design primer. Refer to the primer design principles.
	High primer concentration.	Decrease primer concentration.
	Genome DNA contamination in cDNA template.	To remove genome DNA contamination, treat the RNA sample with DNase (e.g. dsDNase). Or include both upstream and downstream flanking sequences of an intron in one primer.
Poor repeatability	Experimental error.	Use accurate pipettes. Dilute the template to increase volume and reduce error. Use bigger reaction volume.
	Low template concentration	Reduce the dilution ratio of the template.
	Temperature error of the qPCR instrument	Calibrate qPCR instruments periodically.

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