Taq Visual SYBR qPCR Premix (Universal)

REF: EG23115-M/L

Storage Condition

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

Components

Component	EG23115M	EG23115L
Taq Visual SYBR qPCR Premix (Universal)	4×1.25 ml	20×1.25 ml
10× Dilution Buffer	1 ml	5×1 ml

Description

Taq Visual SYBR qPCR Premix (Universal) is a qPCR reagent specifically designed for SYBR Green I intercalating dye-based assays. This 2× premix includes all necessary qPCR components except for primers and DNA samples, thereby reducing operational steps, shortening sample loading time, and minimizing the risk of contamination. Its core component is an antibody-modified hot-start Taq DNA polymerase, combined with a meticulously optimized buffer system and PCR reaction enhancers. This formulation provides strong specificity and high amplification efficiency, effectively inhibiting non-specific amplification and enabling accurate quantification of templates across a wide concentration range, resulting in stable and reliable qPCR results. This product already contains a universal reference dye and is compatible with most qPCR instruments, eliminating the need for additional dyes for instrument calibration.

This product utilizes the color-changing effect resulting from the mixing of different dyes to track the process, thereby significantly minimizing pipetting errors. Taq Visual SYBR qPCR Premix contains a blue dye, while the 10× Dilution Buffer contains a yellow dye. When Taq Visual SYBR qPCR Premix (blue) is mixed with template diluted in Dilution Buffer (yellow), a color change from blue to green occurs, allowing for an accurate visual confirmation of template addition based on the liquid color.

Protocol

1. Template dilution

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

DNA template status	10× Dilution Buffer usage	Dilution Buffer concentration in the template
Solidity	Use ddH_2O to dilute 10 × Dilution Buffer to 1 ×, and dissolve the DNA with the 1× Dilution Buffer.	1 ×
Solution	If necessary, dilute the template to the target concentration with ddH2O, and then add 1 μl 10 × Diffusion Buffer to each 9 μl template.	1 ×

Note: If the Dilution Buffer is used improperly, it may have a certain impact on the qPCR results.

2. Prepare the following mixture in a qPCR tube

Reagent	Amount	Final concentration
Taq Visual SYBR qPCR Premix	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. If Dilution Buffer is used for pipetting tracking (qPCR template includes 1 × Dilution Buffer), the volume of the template should not exceed the range of 2~5 μl/20 μl reaction. If the template usage is less than 2 μl/20 μl reaction, the color will be lighter and affect the tracking effect; if the template usage is higher than 5 μl/20 μl reaction, the active component in the Dilution Buffer may interfere with the qPCR assay. The number of target gene copies contained in different types of DNA templates varies. When necessary, gradient dilution can be performed to determine the optimal amount of DNA template to be added.

Note: The volume of undiluted cDNA template (regardless of whether it contains 1 × Dilution Buffer) should be ≤1/10 of the total volume of qPCR system.

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

2-step PCR

Step	Temperature	Time	
Pre-denaturation	95℃	30 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 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 & Extension or Annealing temperatures according to the Tm of primers. Set Annealing & Extension or Extension time at 15 s for <200bp amplifications. Please also consider the minimum data acquisition time of the instrument used for the time setting.

4. Optimizations

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.

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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.

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.	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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