FS-T-50216 2X Universal Power Plus SYBR Green qPCR Master Mix with UDG

Size: 5 ml / 25 ml

Description	FS-T-50216-5	FS-T-50216-25
2X Universal Power Plus SYBR Green qPCR Master Mix with UDG	5 X 1 mL	25 X 1mL

1 mL develops: 100 reactions (20μl)

***HIGHLIGHTS**:*

Specific—minimize primer-dimer and non-specific amplification

• Reproducible and sensitive—consistent amplification across a wide dynamic range

• Bright—contains SYBR Green for maximum brightness

• Carry-over contamination control—contains heat-labile UDG

• Compatible with all quantitative PCR instruments

Product Description

2X Universal Power Plus SYBR Green qPCR Master Mix contains all the components needed for your real-time PCR reaction, except the template and primers, in a convenient 2X concentration premix designed to be compatible with all types of fluorescence quantitative PCR instruments on the market, including High ROX, Low ROX, and No ROX required instruments.

It utilizes a specially designed reference dye (ROX) for improved sensitivity and resolution. The reagent also incorporates a dUTP/UDG anti-contamination system, which includes UDG to degrade contaminants containing U at room temperature. UDG quickly deactivates when pre-denatured at 95℃ without affecting the efficiency and sensitivity of qPCR. Hot start Taq DNA polymerase is used for amplification, which enhances the specificity of the product while ensuring an efficient amplification effect. Overall, this product provides a reliable and versatile solution for SYBR Green-based qPCR experiments.

Storage

This product should be stored at -20℃ for long-term storage and should be protected from light.

Materials Required

1. EP tubes, PCR tubes and other related materials.
2. qPCR specific primers and templates.
3. qPCR plates and seal membrane.

Instruments

No additional reference dye is required. Universal Power Plus Sybr Green qPCR Mix with UDG is suited for all currently used qPCR instruments (including high ROX mode, low ROX mode and No ROX mode required machine).

qPCR machine Compatibility:

7500 Fast System, 7500 System, QuantStudio™ 12k Flex, QuantStudio™ 3, QuantStudio™ 5, QuantStudio™ 6 Flex, QuantStudio™ 7, StepOne™, Fast Mode, StepOne™, Standard Mode, StepOnePlus™, Fast Mode, StepOnePlus™, Standard Mode, ViiA™ 7 System, AB StepOnePlus™, Fast Mode, AB StepOne™, Standard Mode, AB 7500, Fast Mode, AB 7500, Standard Mode, AB StepOne™, Fast Mode, AB StepOnePlus™, Standard Mode

Usage Notes

1. 2X Universal Power Plus Sybr Green qPCR Mix with UDG, please fully melt it before use, avoid direct sunlight, and store it in a dark place.

2. 2X Universal Power Plus Sybr Green qPCR Mix with UDG contains glycerol, please gently mix before use to avoid bubbles; Mix well and centrifuge before use. After use, immediately return it to the -20℃ refrigerator for storage.

3. This product contains DNA polymerase, so please put it on ice when using it. It can be temporarily stored at 4℃ after multiple uses in a short time. To maintain the quality of the product, it is recommended to avoid repeated freezing and thawing as much as possible.

Operating instructions

Preparation before experiment

1. It is recommended to choose the amplification product length within the range of 70-200 bp.
2. It is recommended to take a reaction volume of 20 μL, add 1 pg-50 ng of DNA as a template, and set NTC (no template control).

To ensure the accuracy of the experimental results, it is recommended to weigh each sample and control group three times.

Experimental methods

Configure qPCR reaction system.

It is recommended to prepare a reaction system on ice and quickly transfer the system to a qPCR instrument preheated at 95 ℃.

20 μL qPCR Reaction

Components	Input
2X Universal Power Plus Sybr Green qPCR Mix with UDG*	10 μL
DNA template *	2 μL
Forward primer（10 μM）**	0.4 μL
Reverse primer（10 μM）**	0.4 μL
ddH2O	To 20 μL

***Note:** Using 10 pg-10 ng **genomic DNA** or 10 pg-100 ng cDNA as the template reference quantity, gradient dilution can be performed on the template to determine the optimal template usage due to the different copy numbers of the target genes contained in the templates of different species. In addition, when using cDNA (RT reaction solution) from the two-step RT qPCR reaction as a template, the addition amount should not exceed 10% of the qPCR reaction system.

****Note:** Typically, the final concentration of the primer is 0.2 μM,and good results can be obtained , and the final concentration of 0.1-1.0 μM can be used as a reference for setting the range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced and the reaction system can be optimized.

1. **Recommended PCR Program**

Step	Temp	Time	Cycles
UDG reaction	37℃	2 min	1
Pre-denaturation	95℃	3 min	1
Cylces	95℃	5 s	40
	60℃	30-34 s *	
Melt Curve		Instrument automatic setting	

**Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30s for StepOne Plus, 31s for 7300 and 34s for 7500.*

Data Analysis

1. To draw a standard curve, use the Ct values of the endogenous gene. The value of R2 should be greater than 0.98 and the slope of curve should be in the range of -3 to -3.5, indicating the PCR amplification efficiency is in the range of 90% to 120%.

2. To ensure reproducibility, the standard deviation (STD) of Ct values should be less than 0.2, and the variation of Ct values for different experiments should be less than 0.5 (with the same threshold value when comparing Ct values).

3. The single melt curve indicates no non-specific amplification products or primer dimmers, and the Tm value in melt curve is usually in the range of 80 to 95℃.

Troubleshooting

1) Multiple Peaks Observed in Melt Curve

a. Check Primer Design: Ensure that the primers were designed following basic primer design protocols.

b. Reduce Primer Concentration: If the concentration of the primers is too high, it may result in non-specific amplification. Lower the concentration of the primers to avoid this issue.

2) Unusual Amplification Curves

a. Non-Smooth Amplification Curve: If the amplification signal is too low, try increasing the template input. Additionally, ensure that the qPCR mix is stored properly.

b. Inconsistent Amplification Curve: Bubbles causes abnormal qPCR results, centrifuge the plate prior to running it.

c. Abnormal Amplification Curves ： the default baseline value of machine is set to be from 3 to 15, the baseline setting can be changed according actual amplification conditions. Besides, the degradation of template may affect the curve,so it is important to ensure the quality of the template before proceeding with the qPCR reaction.

3) No Amplification Curves observed after Reaction

a. Insufficient PCR Cycles: The PCR cycle number is typically set to 40, but if no amplification is observed, it may be necessary to increase the cycle number. Note that a higher cycle number may increase the background signal.

b. Primer Degradation: Check the integrity of the primers using electrophoresis.

c. Confirm Signal Collection Step: The signal collection step is usually set to occur after the annealing-extending step for two-step qPCR, and after the extending step for three-step qPCR. Confirm that this step is set correctly.

d. Insufficient Template Input: If the template concentration is too low, it may not be sufficient for amplification. Try increasing the template concentration or adding an extra repetition.

e. Template Degradation：Use freshly prepared template (Use electrophoresis to confirm Integrity of template)

f. Insufficient Initial Denaturation Time: 2X Univesral Power Plus Sybr Green qPCR Mix uses Hot-Start Taq polymerase, the pre-denaturation time should be at least 3min.

4) Ct Value Too Late

a. Low Amplification Efficiency: Optimize reaction condition or change primer.
b. Insufficient Template Input: Increase template concentration or add extra repeat
c. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm Integrity of template)
d. PCR Products Too Long: The length of amplification products is usually in the range of 70 bp-200 bp. If the products are too long, it may affect the amplification efficiency and result in a late Ct value.

e. PCR Inhibition Reagent: Use new template or dilute the template.

f. Insufficient Pre-denaturation Time 2X Univesral Power Plus Sybr Green qPCR Mix contains Hot-Start Taq polymerase, the pre-denaturation time should be at least 3min.

5) NTC Shows Amplification

a. Contamination: Use sterile water to conduct experiment and the all operation is suggested to be done in clean room to avoid aerosol contamination.

b. Non-Specific PCR Products: analyze with melt curve. If non-specific products are present, troubleshoot the reaction conditions, such as optimizing the primer concentration, changing the annealing temperature, or adjusting the extension time, to reduce the occurrence of non-specific amplification.

6) Inconsistent Results

a. Inconsistent Sample Added: Use proper pipetting techniques.

b. Inconsistent Temperature in qPCR Machine: Ensure periodic machine calibration.

c. Template Concentration Too Low: The lower template input, the poorer qPCR result is. Increase the template concentration.

d. Inconsistent Threshold Set: When comparing the qPCR results in different plates, make sure the threshold value of each experiments is same.

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