BrightCycle Universal SYBR Green qPCR Mix with UDG

Cat. No. : RK21219

Product components

Components	Component number	Size-1 5 mL	Size-2 25 mL
BrightCycle Universal SYBR Green qPCR Mix with UDG *	RM21217	5 × 1 mL	25 × 1 mL

* Including ABclonal Hot Start Taq DNA polymerase, Mg^{2+}, dNTPs, SYBR® Green I, Universal ROX Dye.

Product Description

This product is a universal SYBR Green reagent 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 special reference dye (ROX) for higher sensitivity resolution. BrightCycle Universal SYBR Green qPCR Mix with UDG introduces a dUTP/UDG system preventing the impact of amplification product contamination on qPCR and ensuring the accuracy of the results. UDG can quickly degrade pollutants containing U at room temperature, and 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. 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.

Instruments

No additional reference dye is required. BrightCycle Universal SYBR Green qPCR Mix with UDG is suited for all qPCR instruments (including high ROX mode, low ROX mode and No ROX mode required machine).

Materials Required

1. PCR tubes and other related materials.
2. qPCR specific primers and DNA templates.
3. qPCR 96-well plate and sealing membrane (adhesive film).

Usage Notes

1. BrightCycle Universal SYBR Green qPCR Mix with UDG, please thaw it before use, avoid direct sunlight, and store it in a dark place.
2. BrightCycle Universal SYBR Green qPCR Mix with UDG contains glycerol, please gently mix 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.

Protocol

Before Use

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).
3. To ensure the accuracy of the experimental results, it is recommended to repeat 3 times for each sample and control group.

Experiment procedure

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

Components	Volume
BrightCycle Universal SYBR Green qPCR Mix with UDG *	10 μL
Forward Primer (10 μM) **	0.4 μL
Reverse Primer (10 μM) **	0.4 μL
DNA template	2 μL
ddH_2O	to 20 μL

*** 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.**

**** 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.**

2. Program qPCR reaction as follows:

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: 30 s for StepOne Plus, 31 s for 7300 and 34 s for 7500.**

Data Analysis

1. Draw a standard curve according to Ct values of endogenous gene. The value of R2 should be more than 0.98 and the slope of curve should be in the range of -3 to -3.5 which means the PCR amplification efficiency is in the range of 90% to 120%.
2. The standard deviation (STD) of Ct values should be less than 0.2 and the variation of Ct value for different experiment should be less than 0.5 (the threshold value of different experiments should be same when comparing Ct values).
3. The single melt curve indicate the 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

Melt Curve Show Multiple Peaks

a. Primer Design: Design the primer following basic primer design protocols.

b. Primer Concentration Too High: lower down the concentration of primers.

Unusual Amplification Curves

a. Amplification Curve Not Smooth: Too low amplification signal, increase the template input and make sure 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.

No Amplification Curves after Reaction

a. Not Enough PCR Cycles: the PCR cycle number is usually set to be 40. It should be noted a higher cycle number may increase the background signal.
b. Primer Degradation: Use electrophoresis to confirm the integrity of primers.
c. Confirm the Signal Collection Step: the signal collection step are usually set to be after the annealing-extending step for two-step qPCR and after extending step for Three-step qCPR.
d. Template Input Too Low: Increase template concentration or add extra repetition.
e. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm integrity of template).
f. Not Enough Initial Denaturation Time: BrightCycle Universal SYBR Green qPCR Mix with UDG uses Hot-Start Taq polymerase, the pre-denaturation time should be at least 3 min.

Ct Value Too Late

a. Low Amplification Efficiency: Optimize reaction condition or change primer.
b. Template Input Too Low: Increase template concentration or add extra repeat.
c. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm Integrity of template).
d. Too Long PCR Products: The length of amplification products is usually in the range of 70 bp-200 bp.
e. PCR Inhabitation Reagent: use new template or dilute the template.
f. Too Short Pre-denaturation Time: BrightCycle Universal SYBR Green qPCR Mix with UDG contains Hot-Start Taq polymerase, the pre-denaturation time should be at least 3 min.

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.

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.

Related Products

Name	Catolog	Size
ABScript Neo RT Master Mix for qPCR	RK20432	10 RXN / 100 RXN
ABScript Neo RT Master Mix for qPCR with gDNA Remover	RK20433	10 RXN / 100 RXN
ABScript II cDNA First-Strand Synthesis Kit	RK20400	50 RXN / 100 RXN