Instructions for Realtime PCR Fluorescent Quantification Kit (SYBR Green I)

Item No. : SR1110
Specification: 50T (1.25mL) /200T (4 x 1.25mL)
Storage: This product is valid for at least one year at -20℃, and can be stored for 3 months at 2-8℃. After thawing, it should be thoroughly mixed to avoid a large number of bubbles.
Reagent composition: 2 x SYBR Green PCRmix contains PCR Buffer, $MgCl_2$, dNTPs, HS Taq DNA Polymerase, SYBR Green I, stabilizer and other ingredients.

Product introduction:

2×SYBR Green PCRmix is a special reagent for Real Time PCR using SYBR Green I chimeric fluorescence method. The hot initiation of HS Taq DNA Polymerase by chemical modification containing complete block of Taq enzyme activity at room temperature can effectively inhibit the non-specific amplification caused by primer non-specific annealing or primer dimer at low temperature, and improve the specificity of amplification reaction. The reagent is specially prepared by using an optimized qPCR Buffer, which greatly improves the amplification efficiency and detection sensitivity of the qPCR reaction, and can obtain a good standard curve in a wide quantitative area for accurate quantification. This reagent is compatible with fluorescent quantitative PCR instruments from many manufacturers, such as Applied Biosystems, Eppendorf, Bio-Rad and Roche.

Principle of the reagent:

The polymerase HS Taq DNA polymerase was chemically modified for qPCR amplification. The fluorescence signal intensity of the polymerase was detected by chimeric SYBR Green I.

1. PCR

PCR method is a method to amplify the target fragment of trace DNA. A large number of DNA fragments can be amplified in a short time by repeating three steps: thermal denaturation of DNA strand, annealing of primer and extension of primer under the action of DNA polymerase.

2. Fluorescence detection

SYBR Green I is a double-stranded DNA binding dye that binds to small furrows. When combined with double-stranded DNA, its fluorescence is greatly enhanced, with a maximum absorption wavelength of about 497nm and a maximum emission wavelength of about 520nm.

SYBR Green I can be combined with all double-stranded DNA without the use of a probe, and can be detected with good versatility and high sensitivity. However, because SYBR Green I binds to all double-stranded DNA, false positives caused by primer dimers, single-stranded secondary structures, and faulty amplification products can affect the accuracy of quantification. In the process of quantitative instrument detection, the uniformity of products can be analyzed by measuring the change of fluorescence after increasing temperature, so as to distinguish the melt peak temperature of products and distinguish the specific and non-specific products.

How to use:

Reaction conditions

Reaction conditions 1 Two-step method

Hot start: 95℃ for 10 minutes;

Variability: 95℃ 10~20 seconds;

Annealing/extension: 60℃ 20~60 seconds. } 35~45 cycles

Analysis of melting curve.

2. Three-step method

Hot start: 95℃ for 10 minutes;

Variable: 95℃ 10~20 seconds;

Deheat: 56-64℃ for 10 to 30 seconds; } 35~45 cycles

Elongation: 72℃ 10~60 seconds.

Analysis of melting curve.

For Roche LightCycler480, the hot start time should be 10min, ABI7500 can also be 5min hot start.

The qPCR reaction system is formulated

reagent	25μL system	50μL system	Final concentration
2×SYBR Green PCRmix	12.5 μL	25 μL	1 x
Primer 1 (10μM)	0.5 to 2.5 μL	1-5 μL	0.2 ~ 1.0 microns
Primer 2 (10μM)	0.5 to 2.5 μL	1-5μL	0.2 ~ 1.0 microns
Template DNA	5 μL	10 μL	-
ddH_2O	-	-	-
Total volume	25 μL	50 μL	

What to watch for:

1, 2×SYBR Green PCRmix is specially formulated with chemically modified hot start enzyme, which has higher specificity;

2、For primers with low annealing temperature or long fragments over 200bp amplification, three-step method is recommended;

3, before and after amplification to use a special area and pipette, wear gloves to operate and often replace, do not open the reaction tube after PCR reaction is completed. To minimize the contamination of PCR products to the sample.

Related products:

SR2110 2×Taqman PCR MasterMix

SR4110 SYBR Green I(PCR grade)

PC2200 dNTPs Mix(10mM each)

Related literature:

[1] Baoling Ju,Ying Nie,Xufang Yang,et al. miR-193a/b-3p relieves hepatic fibrosis and restrains proliferation and activation of hepatic stellate cells. Journal of Cellular and Molecular Medicine. April 2019. (IF 4.658)
[2] Wenlin Tai,Shuhao Deng,Wenjuan Wu,et al. Rapamycin attenuates the paraquat-induced pulmonary fibrosis through activating Nrf2 pathway. Journal of Cellular Physiology. July 2019. (IF4.522)
[3] Liu Lin,Yu Ye,Xiumei Zhu. MMP-9 secreted by tumor associated macrophages promoted gastric cancer metastasis through a PI3K/AKT/Snail pathway. Biomedicine & Pharmacotherapy. September 2019. (IF3.743)

Note: Please refer to the official website of Solarbio for more literature on the use of this product.