2×taqman PCRmix (Probe qPCR)

Item number: SR2110

Specification: 50T (1.25mL) /200T (5mL)

Storage: Store at -20 °C. After thawing, mix well before use to avoid a lot of air bubbles.

Product Description:

2×taqman PCRmix (Probe qPCR) is a special reagent for Real Time PCR using the probe method. HS Taq DNA Polymerase is thermally-activated by a chemical modification containing complete block of Taq enzyme activity at room temperature, which can effectively inhibit 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:

2×taqman PCRmix (Probe qPCR) uses the chemically modified hot-start enzyme HS Taq DNA polymerase produced by our company to perform qPCR amplification, and completely blocks the activity of Taq enzyme at room temperature. Therefore, the reaction system can be prepared at room temperature. taqman probe was used to detect the fluorescence signal intensity of PCR amplification.

Reagent composition:

1, 2 x taqman PCRmix (Probe qPCR)

2, ROX Reference Dye I (50×)

3, ROX Reference Dye II (100×)

ROX Reference Dye is used to correct the interpore fluorescence signal error of some Real Time PCR amplifiers of our company.

ROX Reference Dye I (50×) is suitable for:

ABI PRISM 7000/770/7300/7900HT, StepOnePlus Real-Time PCR System.

ROX Reference Dye II (100 x) is suitable for:

7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, Stratagene Mx3000P, Mx3005P, Mx4000.

The final reaction concentration of ROX Reference Dye I and II was 1×.

LightCycler, Thermal Cycler Dice Real Time SystemII, Smart Cycler System and other Real Time PCR amplifiers are not required.

Reaction conditions:

1. Two-step method

Hot start: 95℃ for 3 minutes;
Variability: 95℃ 10~20 seconds;
Annealing/extension: 60℃ 20~60 seconds. } 35~45 cycles

2, three step method
Hot start: 95℃ for 3 minutes;
Variable: 95℃ 10~20 seconds;
Deheat: 56-64℃ for 10 to 30 seconds; } 35~45 cycles
Elongation: 72℃ 10~60 seconds.

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

qPCR reaction system is formulated:

reagent	25μL system	50μL system	Final concentration
2×taqman PCRmix	12.5μL	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	1-5 μL	0.2 ~ 1.0 microns
TaqMan Probe	1 μL	2 μL	-
ROX Reference Dye I or ROX Reference Dye II	0.5μL or 0.25μL	1μL or 0.5 μL	1 x
Template DNA	5 μL	10 μL	-
ddH_2O	-	-	-
Total volume	25 μL	50 μL	

1、Good results are usually obtained with a final primer concentration of 0.2μM. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2 ~ 1.0μM.

2、The probe concentration used can be optimized in the range of 0.1-0.3μM. Experiments with concentration gradients can be performed to find the best combination of primers and probes. The use of the probe is related to the Real Time PCR amplification instrument, the type of probe, and the type of fluorescent labeled substance. Please refer to the instrument instruction manual or the specific use requirements of each fluorescent probe.

3、Different types of DNA templates contain different copy numbers of target genes, and gradient dilution can be carried out if necessary to determine the optimal amount of DNA template addition.

Quality control:

1. Functional test: sensitivity, specificity and repeatability of qPCR.

2. No external ribonuclease activity, no endonuclease activity, no exonuclease deoxyribonuclease contamination.

Technical description:

1, 2×taqman PCRmix is specially formulated, using chemical modification of hot start enzyme, with higher specificity;

2、The three-step method is recommended for primers with low annealing temperature or longer fragments over 200bp;

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.