SIBER Q-PCR Master Mix

ROX-step

Cat. No. : DA0110-0001

Concentration : 2X

Volume : 1 ml

Storage : -20 ℃ protected from light.

Description

The SYBR Green Premix is a ready-to-use, 2X concentrated master mix reagent including Hotstart Taq and SYBR Green I, specially designed for real-time PCR with intercalator method.

Content

2X SYBR Green Premix containing:

- Hotstart Taq DNA polymerase
- SYBR Green real-time PCR Buffer
- dNTP mix including dATP、dCTP、dGTP、dTTP
- 5mM $MgCl_2$

Procedure

A. Preparation of the PCR Mix

1. Preparing a master mix as follows:

Component	Volume / reaction	Final conc.
2x SYBR Green Premix	12.5 μl	
Forward Primer (10 μM)	variable	0.3~1.0 μM
Reversed Primer (10 μM)	variable	0.3~1.0 μM
H_2O	upto 23.0 μl	

2. Mix the master mix thoroughly by pipetting up and down.
3. dispense 23 μl volumes into PCR tubes or plates.
4. Add 2 μl of the DNA or cDNA, Mix carefully by pipetting up and down.

Performing PCR

1. Program your instrument as follows:

Step	Time	Temperature
Initial PCR activation step	10 min	95 ℃
2-step cycling Denaturation	15~30 sec	94 ℃
Annealing /Extension	30~60 sec	50-68 ℃
Cycle number	35~45 cycles	
Optional:		
Data acquisition	15 sec	X* ℃

＊Tm dimmer ＜ X ＜ Tm product

2. Place the PCR tubes or PCR plates in the thermal cycler and start the cycling program.
3. Perform a melting curve analysis of the PCR products.

SYBR Green Premix performance test

- Consistently high specificity over a broad dynamic range. Ten fold serial dilution (10^9~10^0) of plasmid DNA were amplified using primers specific to the NNV gene, Triplicate reactions at each concentration were amplified along with no- template controls. Standard curve had r=0.999, efficiency=92.1%, Standard deviation of Ct ＜1.0.

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