SYBR Green qPCR Mastermix

User's Instruction

Description

SYBR Green qPCR Mastermix is a ready-to-use mastermix which provides superior specificity, robust amplification efficiency, ideal reproducibility and stability in quantifying target DNA or cDNA, with minimal level of primer-dimer and non-specific product formation. This mastermix takes advantage of a novel chemically-modified hot-start Taq polymerase combined with the antibody. The polymerase activity is inhibited at temperatures below 40°C reversibly. SYBR Green I in the mix will emit fluorescence when binds to the dsDNA amplified in each cycle. This reagent is without ROX Reference Dye in default, but can be supplied together with low or high ROX Reference Dye if required. When conducting the experiment, use the SYBR® or SYBR/FAM scan mode setting on the qPCR instrument.

Feature

- **High specificity:** Optimized buffer and reversible inhibition can achieve hot-start effect in whole reaction.
- **High efficiency:** The Ct value increases earlier than similar products on the market.
- **High compatibility:** This mastermix has been verified on most mainstream qPCR instruments.

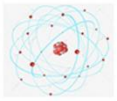
Kit Contents

1. SYBR Green qPCR Mastermix (2×)	5 ml

Protocol

1. Set up the qPCR reaction on ice as the following table:

Reagent	Volume	Final Concentration
SYBR Green qPCR Mastermix (2×)	12.5 μL	1×
Forward Primer (10 μM)	0.5 μL	0.2 μM [1*]
Reverse Primer (10 μM)	0.5 μL	0.2 μM [1*]
Template (DNA)	1 μL	-
ddH_2O	up to 25 μl	-



2. Mix the reaction system (gently blow several times with a pipette to fully mix or gently mix with a vortex mixer at a lower speed), and then centrifuge to precipitate the liquid.

Thermocycling Conditions for qPCR (Two-Step)

1. Hot Start: 94°C for 2-3 min
2. 40 cycles:
    a) 94°C for 5-10 sec
    b) 60°C for 30-35 sec
3. Melting curve: default

Thermocycling Conditions for qPCR (Three-Step)

1. Hot Start: 94°C for 2-3 min
2. 40 cycles:
    a) 94°C for 10-20 sec
    b) 55-60°C for 10-20 sec
    c) 72°C for 20-30 sec
3. Melting curve: default

Note: *Generally, two-step method has higher specificity while three-step method has higher efficiency. If melting curve is not very good, we recommend to use two-step method. If using primers with low Tm value and resulting in bad result, then we recommend to extend the extension time or use three-step method.*

Storage

Minimum shelf life is 6 months at -20°C with light-free.

Only for research and not intended for treatment of humans or animals