2×SYBR Green PCR Mastermix

Cat No.: SLPCR230

Package: $50T(1.25ml)/200T(4\times1.25ml)$ **Storage:** $-20^{\circ}C$, valid for at least one year.

Component

1. 2×SYBR Green PCRmix: PCR Buffer, MgCl₂, dNTPs, HS Taq DNA Polymerase, SYBR Green I, stabilizer.

2. ROX Reference Dye I(50×)

3. ROX Reference Dye II(100×)

ROX Reference Dye $I(50\times)$ application for:

ABI PRISM 7000/7700/7300/7900HT, Step One Plus Real-Time PCR System.

ROX Reference Dye II(100×) application for:

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

Final Concentration of ROX Reference Dye I, II is 1×.

LightCycler, Thermal Cycler Dice Real Time SystemII, Smart Cycler System need no ROX.

Product Description

2×SYBR Green PCR Mastermix a ready-to-use reaction cocktail that contains all components, except primers and template, for real-time quantitative PCR (qPCR) systems. This unique combination of proprietary buffer, stabilizers, and HS Taq DNA Polymerase delivers maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional cycling protocols with SYBR Green qPCR. Highly specific amplification is crucial to successful qPCR with SYBR Green I dye technology because this dye binds to and detects any dsDNA generated during amplification. HS taq DNA polymerase has no activation at low temperature, so it can inhibits the extension of nonspecific primers and the formation of primer dimmers.

The product has been optimized for use with all currently available real-time cyclers, such as Applied Biosystems, Eppendorf, Bio-Ra, Roche etc., The ROX Reference Dye is provided in a separate tube and can be added if using a cycler that requires ROX as a passive reference dye.

Reaction Assembly

| Component | 25μl Volume | 50μl Volume | Final Concentration |
|----------------------------|-------------|-------------|---------------------|
| 2×SYBR Green PCR Mastermix | 12.5μl | 25µl | 1× |
| Primer 1(10μM) | 0.5-2.5μ1 | 1-5µl | 0.2~1.0μM |
| Primer 2(10μM) | 0.5-2.5μ1 | 1-5µl | 0.2~1.0μM |
| Template DNA | 5μl | 10µl | - |
| ddH2O | - | - | - |
| Total volume | 25μl | 50µl | |

PCR Cycling Protocol

2-Step Cycling:

| pre-denaturation | Denature | Anneal/ Extend | |
|------------------|--------------|----------------|--|
| 1 CYCLE | 35–45 CYCLES | | |
| 95°C | 95°C | 60°C | |
| 10 min | 10~20s | 20~60s | |

Melt Curve (dissociation stage)

3-Step Cycling:

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|------------------|------------------|--------------|--------|--------|--|--|--|
| | pre-denaturation | Denature | Anneal | Extend | | | |
| | 1 CYCLE | 35–45 CYCLES | | | | | |
| | 95°C | 95°C | 56-64℃ | 72℃ | | | |
| | 10 min | 10~20s | 10~30s | 10~60 | | | |

Melt Curve (dissociation stage)

Note:

- 1. For the Roche LightCycler480, the pre-denaturation time suggest 10min; ABI7500 suggest 5min.
- 2. If the annealing temperature is lower or exceed 200bp, please use the three-step method.