

User Manual

Version 2.0

Product name: 2X HotSybr Real-time PCR Kit (Regular ROX)

Cat #: HSM400

Description:

Contains regular ROX. For ABI 7000, 7300, 7700, 7900 machines.

A key component of the 2X HotSybr Real-time PCR Kit is a stringent hot-start DNA polymerase. This proprietary technology ensures robust and highly sensitive PCR amplification and quantification.

Storage:

Store at -20°C . To avoid repeated freeze-thaw, opened vials should be kept at 4°C .

Primer design:

To achieve the best performance, appropriate software, such as Primer Express, should be used.

1. T_m : $58\sim 60^{\circ}\text{C}$ with 17 ~ 30 nucleotides in length
2. To design a small amplicon, <150bp
3. To avoid secondary structures
4. To avoid more than 3 consecutive Gs
5. To avoid complementary 3' ends

Recommended thermocycling condition:

95°C , 10 min. => (95°C , 5 sec. => 60°C , 30 sec.) for 40 cycles => melting curve.

Tips for good performance:

Primer concentration should not be high. 100nM to 300nM of each primer usually gives the best results. Melting curve is a useful way to distinguish non-specific amplification products and primer dimers from the desired product. It is recommended to run melting curve following amplification cycles.

To do replicates.

To reduce pipetting errors: preparing an amplicon specific master mix, using a repeat pipet, and keeping pipetting volumes within the manufacture's suggested range are very effective to get a tight Ct.