MCLAB
Molecular Cloning Laboratories

User Manual

Version 2.0

Product name: 2X HoTaq Real-time PCR Kit (ROX free)

Cat #: HTP400RF

Description:

Contains NO ROX. For BioRad iCycler MiniOpticon, Opticon 2, Chromo4, iQ5; Roche LightCycler 480; MJ Research DNA Engine Opticon 2, Chromo4; Corbett Roto-gene 3000, 6000 machines.
This product enables sensitive detection of DNA and fast thermocycling, using proprietary hot-start PCR technology developed at MCLAB (patent pending).

Storage:

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

Primer and probe design:

To achieve the best performance, appropriate software, such as ABI's Primer ExpressTM, should be used.
1. Tm: 60˚C for primers and 68~70˚C for probes
2. Amplicon size should be small, <150bp
3. To avoid secondary structures and avoid more than 3 consecutive Gs in primers and probes
4. Primers should be 17 ~ 30 nucleotides in length and should not have complementary 3' ends

Reaction conditions:

95˚C, 10 min. => (95˚C, 5 sec. => 60˚C, 30 sec.) for 50 cycles.

Tips for good performance

To achieve accurate quantification, it is highly recommended to do replicates. Three is the minimal number of replicates to obtain a standard deviation. It is important to reduce pipetting errors. Three ways to minimize pipetting errors are:
1. To prepare an amplicon specific master mix that includes PCR reaction mix, primers, and probes.
2. To use a repeat pipet.
3. To pipet volumes within the manufacture's suggested range.