



## 2X HoTaq PCR Reaction Mix

CAT # (SKU): HTP-400

FOR RESEARCH USE ONLY

### Storage

This product should be stored at  $-20^{\circ}\text{C}$ . To avoid repeated freeze-thaw, opened vials should be kept at  $4^{\circ}\text{C}$ .

### About the product

2X HoTaq PCR Reaction Mix utilizes a proprietary hot-start PCR technology (patent pending). It enables sensitive detection of DNA and fast thermocycling.

### Primer and probe design

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

1.  $T_m$ :  $60^{\circ}\text{C}$  for primers and  $68\sim 70^{\circ}\text{C}$  for probes
2. Amplicon size should be small,  $<150\text{bp}$
3. To avoid secondary structures in primers and probes
4. To avoid more than 3 consecutive Gs in primers and probes
5. Primers should not have complementary 3' ends
6. 17 ~ 30 nucleotide in length

### Reaction conditions

The recommended thermocycling condition is:  $95^{\circ}\text{C}$ , 10min.  $\rightarrow$  ( $95^{\circ}\text{C}$ , 5 sec.  $\rightarrow$   $60^{\circ}\text{C}$ , 30 sec.) for 50 cycles.

### Notes

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 error. Three ways to minimize pipetting error 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 volume within manufacture's suggested range.

This reaction mix contains internal passive reference dye (ROX).