

## Real HotStart DNA Polymerase Mastermix

### Ultra-High Sensitivity



Store at  
-20°C

#### Cat. No. RT101

**250 units, without loading dye**

Reaction: 100 Reactions

2X Real HotStart DNA Polymerase Mastermix  
(200 U/ml): 1.25 ml

Control DNA Template (1 x 10<sup>-14</sup> g): 3 µl

Control Primer Mix (5 µM): 4.5 µl

Sterile Deionized Water: 1.5 ml

#### Cat. No. RT102

**250 units, with loading dye**

Reaction: 100 Reactions

2X Real HotStart DNA Polymerase Mastermix  
(200 U/ml): 1.25 ml

Control DNA Template (1 x 10<sup>-14</sup> g): 3 µl

Control Primer Mix (5 µM): 4.5 µl

Sterile Deionized Water: 1.5 ml

## Description

Real HotStart DNA Polymerase Mastermix is an ultra-sensitive PCR product. It works excellent especially for short DNA template (size shorter than 600 bp). It contains all the factors and loading dye\* needed to perform PCR, including HotStart DNA polymerase, dNTPs, 7.5 mM MgCl<sub>2</sub>, loading dye\*(including bromphenol). The only step to perform PCR is to add the template and primers into the tube containing RealStart DNA Polymerase Mastermix. Since the special HotStart DNA polymerase in Real HotStart DNA Polymerase Mastermix activates only after heating, it also reduces the risk of contamination when working with Real HotStart DNA Polymerase Mastermix at room temperature. Real HotStart DNA Polymerase Mastermix makes PCR simple and easy, eliminating the extra handling steps and contamination risks associated with conventional hot-start methods. \*optional

## Storage Condition

Real HotStart DNA Polymerase Mastermix should be stored immediately upon receipt at -20°C in a constant temperature freezer.

## Notes

- 1 Real HotStart DNA Polymerase Mastermix requires an activation step of 15 minutes at 95°C.
- 2 Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- 3 Use sterile and filtrated ddH<sub>2</sub>O to eliminate possible contaminations.
- 4 This protocol serves only as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperature, and amount of template DNA may vary and must be individual determined.
- 5 For research use only. Note for use in diagnostic or therapeutic procedures.

## General Reaction Conditions

1. Add the following components to a sterile microtube on ice:

Components	Volume	Final Concentration
Real HotStart DNA Polymerase Mastermix (0.2 U/µl)	12.5 µl	-
Forward Primer (10 µM)	0.75 µl	0.3 µM
Reverse Primer (10 µM)	0.75 µl	0.3 µM
Template DNA	2 µl	-
Sterile Deionized Water	to 25 µl	-

2. Suggested Reaction Parameters for Real HotStart DNA Polymerase Mastermix.

Segment	Number of cycles	Temperature	Duration
1	1	95°C	15 minutes
2	35~45	94°C (Denature)	30 seconds
		50~68°C (Anneal)	30 seconds
		72°C (Extend)	30 seconds~1 minute
3	1	72°C	1 minute
		4°C	

\* For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.

3. Add Positive Control according to following components:

Components	Volume	Final Concentration
Real HotStart DNA Polymerase Mastermix (0.2U/µl)	12.5 µl	-
Control Primer Mix (5 µM)	1.5 µl	0.3 µM
Control DNA Template (1 X 10 <sup>-14</sup> g)	1 µl	-
Sterile Deionized Water	to 25 µl	-

\* Program the thermal cycler according to step 2 but change the annealing temperature to 54°C and number of cycle to 40.

\* The length of PCR product is 450 bp.

Recombinant	✓
5' to 3' Exonuclease	✓
3' to 5' Exonuclease	✗
Terminal dA Addition	✓
Endonuclease Free	✓