

RealSens™ HotStart DNA Polymerase Mastermix

Ultra-High Sensitivity

Store at
-20°C

Cat. No. RT101

12.5 µl x 100 reactions; without loading dye
2X RealSens™ HotStart DNA Polymerase Mastermix: 1.25ml

Cat. No. RT141

12.5 µl x 400 reactions; without loading dye
2X RealSens™ HotStart DNA Polymerase Mastermix: 5ml

Cat. No. RTD101

12.5 µl x 100 reactions; with loading dye
2X RealSens™ HotStart DNA Polymerase Mastermix: 1.25ml

Cat. No. RTD141

12.5 µl x 400 reactions; with loading dye
2X RealSens™ HotStart DNA Polymerase Mastermix: 5ml

Unit Definition

One Unit of enzyme catalyzes the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 72°C.

Description

RealSens™ HotStart DNA Polymerase Mastermix is supplied as 2-fold concentrated, ready-to-use mixture. RealSens™ HotStart DNA Polymerase Mastermix is an ultra-sensitive PCR product which works excellent for short DNA template (size shorter than 600bp). It contains all the factors needed for performing PCR, including HotStart DNA polymerase, dNTPs, MgCl₂ and loading dye (optional). The only step to perform PCR is to add the template and primers into the tube containing 2X RealSens™ HotStart DNA Polymerase Mastermix.

Since the HotStart DNA polymerase in RealSens™ HotStart DNA Polymerase Mastermix activates only after heating, it also reduces the risk of contamination when working with RealSens™ HotStart DNA Polymerase Mastermix at room temperature. RealSens™ HotStart DNA Polymerase Mastermix makes PCR simple and easy, eliminating the extra handling steps and contamination risks associated with conventional hot-start methods.

Features

Ultra-high sensitivity equivalent to real-time PCR. Less non-specific amplification error. Ideal for T&A Cloning Kit (RC001).

Applications

High throughput hot-start PCR, RT-PCR, highly specific amplification of complex genomic and cDNA templates, amplification of low copy DNA targets, generation of PCR products for TA cloning.

Storage Condition

RealSens™ HotStart DNA Polymerase Mastermix should be stored immediately upon receipt at 20°C in a constant temperature freezer. Avoid repeated freeze-thaw cycles.

General Reaction Conditions

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

Components	Volume	Final Concentration
RealSens™ HotStart DNA Polymerase Mastermix	12.5 µl	1X
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 RealSens™ 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 4°C	1 minute

* 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
RealSens™ HotStart DNA Polymerase Mastermix	12.5 µl	-
Control Primer Mix (5 µM)	1.5 µl	0.3 µM
Control DNA Template (1 X 10 ⁻¹⁴ 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.



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