ealSens™ HotStart DNA Polymerase **Ultra-High Sensitivity** 

#### Cat. No. RTH01 500 units, with dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 100 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 1ml 10mM dNTPs Mix: 200µl

# Cat. No. RTH02

500 units, without dNTP
Real Sens™ HotStart DNA Polymerase (5U/µl): 100 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 1ml

### Cat. No. RTH11 1000 units, with dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 200 ul

10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 2ml 10mM dNTPs Mix: 400µl

#### Cat. No. RTH22 1000 units, without dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 200 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 2ml

## Storage Buffer

20 mM Tris-HCl (pH 8.0), 50% (v/v) glycerol, 0.5%NP-40, 0.5%Tween 20, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT.

### 10X Reaction Buffer

100 mM Tris-HCl (pH 9.0), 500 mM KCl, 0.1% (w/v) gelatin, 20 mM MgCl $_2$ , 1% Triton X-100.

## **Description**

RealSens™ HotStart DNA Polymerase is ideal for DNA fragment amplification, especially for short DNA template (size shorter than 600bp). Since RealSens™ HotStart DNA Polymerase activates only when temperature reaches 95°C for 10 minutes, it pervents the formation of misprimed products and primer-dimers at low temperature during PCR setup and the initial PCR cvcle.

No additional heating step for polymerase activation is required! RealSens™ HotStart DNA Polymerase activates during the first denaturation step. It's especially designed for high PCR specificity with minimal optimization and nonspecific amplification. It makes PCR setup simple and easy while all components could be assembled at room temperature.

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 clonina.

## **Unit Definition**

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

## **Storage Condition**

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

## **General Reaction Conditions**

The optimal conditions for the concentration of RealSens™ HotStart DNA Polymerase, primers and template DNA depend on the system being ultilized. It may be necessary to determine the optimal conditions for each indivisual component.

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

| Components                               | Volume         | Final Concentration |
|--|----------------|---------------------|
| 10X Reaction Buffer                      | 2.5µl          | 1X                  |
| 10 mM dNTP mix                           | 2.5µl          | 0.1 mM              |
| Primer mix (10 μM each)                  | 1.5µl          | 0.3μΜ               |
| Template DNA                             | 2.0μΙ          | n/a                 |
| RealSens™ HotStart DNA Polymerase(5U/uI) | 0.5 μΙ         | 2.5 units           |
| D.W.                                     | Add to 25.0 μl | n/a                 |

2.Suggested Reaction Parameters

| Segment | Number of Cycles | Temperature    | Duration   |
|---------|------------------|----------------|------------|
| 1       | 1                | 95℃            | 10 minutes |
|         |                  | 94℃ (Denature) | 30 seconds |
| 2 40    | 50~68℃ (Anneal)  | 30 seconds     |            |
|         | 72℃ (Extend)     | 30 seconds     |            |
| 3       | 1                | 72℃            | 1 minute   |

3.Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining

**Note** For research use only. Not for use in diagnostic or therapeutic procedures.



Real Biotech Corporation

www.real-biotech.com

-20°C

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# Store at Cat. No. RTH01 500 units, with dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 100 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 1ml 10mM dNTPs Mix: 200µl

### Cat. No. RTH02 500 units, without dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 100 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 1ml

## Cat. No. RTH11 1000 units, with dNTP

Real Sens™ HotStart DNA Polymerase (5U/µl): 200 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>):2ml 10mM dNTPs Mix: 400ul

# Cat. No. RTH22

1000 units, without dNTP Real Sens™ HotStart DNA Polymerase (5U/µl): 200 ul 10X Reaction Buffer (with 20 mM Mg<sup>2+</sup>): 2ml

# Storage Buffer

20 mM Tris-HCl (pH 8.0), 50% (v/v) glycerol, 0.5%NP-40, 0.5%Tween 20, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT.

## **10X Reaction Buffer**

100 mM Tris-HCl (pH 9.0), 500 mM KCl, 0.1% (w/v) gelatin, 20 mM MgCl<sub>2</sub>, 1% Triton X-100.

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## **Features**

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

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| Primer mix (10 μM each)                  | 1.5µl          | 0.3μΜ               |
| Template DNA                             | 2.0μΙ          | n/a                 |
| RealSens™ HotStart DNA Polymerase(5U/ul) | 0.5µl          | 2.5 units           |
| D.W.                                     | Add to 25.0 μl | n/a                 |

2.Suggested Reaction Parameters

| Segment | Number of Cycles | Temperature      | Duration   |
|---------|------------------|------------------|------------|
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3.Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining

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