GenScript *RealStart Taq* DNA Polymerase E00041

Technical Manual No. 0261 **Version 20080120**

I. INTRODUCTION

GenScript *RealStart Taq* DNA Polymerase is designed for highly specific PCR, such as those performed with the Hot-Start PCR technique, which enhances the specificity, sensitivity, and yield of PCR DNA amplification(1,2). In general, Hot-Start PCR methods reduce or eliminate non-specific and unwanted DNA amplification, such as that of primer-dimers, and other artifacts during PCR reaction set-up and before real PCR starts. GenScript *RealStart Taq* DNA polymerase has been engineered and formulated to function only in true PCR cycles, eliminating non-specific amplification.

GenScript *RealStart Taq* DNA Polymerase is formulated in such a way that **no prolonged heating or denaturing step is necessary,** making *RealStart Taq* polymerase more convenient and easy to use.

II. APPLICATIONS

The applications of GenScript *RealStart Taq* DNA Polymerase include the following:

- High-specificity PCR
- Amplification of complex genomic or cDNA templates
- Amplification of DNA targets at low concentrations
- Multiplex PCR
- 3’ A-tailing of blunt-end DNA

III. CONCENTRATION AND UNIT DEFINITION

The product is supplied at a concentration of 5 units/µl. One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble fraction in 30 minutes at 74°C.

IV. BUFFERS

RealStart Taq DNA Polymerase Storage Buffer is formulated as follows: 20 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.01% Triton X-100, 50% glycerol.

Two 2X Reaction Buffers are included: 2X Reaction buffer I (with Mg^{2+}) and 2X Reaction Buffer II (with Mg^{2+}). Always use 2X Reaction Buffer I (with Mg^{2+}) first. In some rare cases, 2X Reaction Buffer I may not work, then use 2X Reaction Buffer II.

V. SHIPPING AND STORAGE

Store the product at -20 °C. The enzyme remains stable for at least 12 months if stored at -20°C. This product is shipped on blue ice.

VI. GENERAL PCR PROTOCOL

This is a protocol to be used as a general guide. Optimization may be needed in some cases.

1. Thaw all the reagents on ice. Vortex to mix well and then spin down briefly.

   Set up 50 µl PCR reaction in a thin-walled PCR tube on ice:

25.0 µl	2X *RealStart Taq* Buffer I
1.0 µl	10 mM dNTP stock
1.0 µl	forward primer (10 µM)
1.0 µl	reverse primer (10 µM)
1.0 µl	template (up to 100 ng/µl)
20.5 µl	PCR-grade H_2O
0.5 µl	*RealStart Taq* Polymerase (5 units/µl)

2. Program PCR cycler as follows:

Initial denaturing:	94°C for three minutes
Then 30-35 cycles of:	94 °C for 30 seconds Tm - 5 °C for 30 seconds 72 °C for 60 seconds (about 1 kb/minute)
Extension:	72 °C for five minutes
End of PCR:	Cool down to room temperature or to 4°C.

3. When the temperature of PCR cycler reaches 94°C, put PCR reaction tube in and start the program.
4. Analyze PCR fragments on an agarose or polyacrylamide gel after PCR reaction.

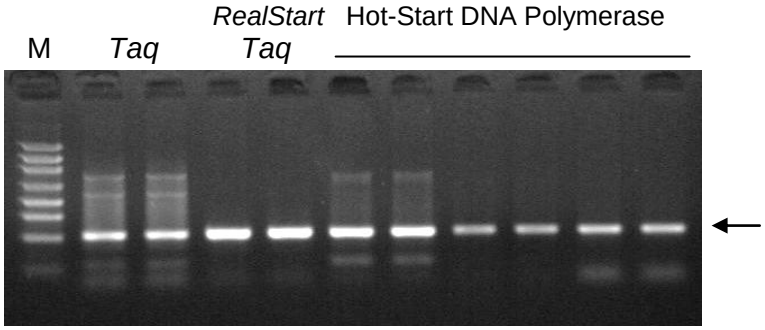
Note:

1. Annealing temperature should be 5°C below the calculated T_m of the primers.
2. This protocol is for PCR cycler with a hot lid. With some other models, mineral oil must be added to prevent evaporation.

VII. EXAMPLES

Shown below is a comparison of *RealStart Taq* DNA Polymerase to other vendors' Hot-Start DNA polymerases.

The PCR reactions were set up using *Taq*, *RealStart Taq* and Hot-Start DNA polymerases from vendors A, B, and C, respectively. The expected PCR DNA is denoted by an arrow.



VII. TROUBLESHOOTING

Use the chart below to solve and avoid common problems.

Non-specific bands	
The annealing temperature is too low.	Raise the annealing temperature in increments of 5°C.
The extension time is too short.	Lengthen the extension time in increments of 30 seconds.
The cycle number is too high.	Reduce the number of cycles in decrements of two cycles.
The primer design is not appropriate.	Redesign the primers with higher specificity to the target DNA.
No product or poor amplification yield	
The denaturation time is too long.	Longer denaturation times may damage the DNA template.
The enzyme concentration is too low.	Increase the concentration of enzyme in increments of 0.25 U.
The cycle number is too low.	Increase the number of cycles.
The annealing temperature is too high.	Lower the annealing temperature in increments of 2°C.
The annealing time is too short.	Increase the annealing time in increments of 20 seconds.
The extension time is too short.	Increase the extension time in increments of 30 seconds.
Buffer I is not appropriate here.	Use Buffer II for PCR.

IX. ORDERING INFORMATION

RealStart Taq DNA Polymerase, Cat. No. E00041

GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-357-3839
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com