# SureStart *Taq* DNA Polymerase

## Instruction Manual

**Catalog #600280 (100 U), #600282 (500 U), #600284 (1000 U)**

Revision C.0

**For Research Use Only. Not for use in diagnostic procedures.**

600280-12

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# SureStart *Taq* DNA Polymerase

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# SureStart *Taq* DNA Polymerase

## MATERIALS PROVIDED

| Material Provided | Quantity[a] | | |
|---|---|---|---|
| | #600280 | #600282 | #600284 |
| SureStart *Taq* DNA polymerase (5 U/µl) | 100 U | 500 U | 1000 U |
| SureStart *Taq* 10× reaction buffer | 1 ml | 2 × 1 ml | 4 × 1 ml |

[a] Catalog #600280 provides sufficient reagents for 80 × 50-µl reactions. Catalog #600282 provides sufficient reagents for 400 × 50-µl reactions. Catalog #600284 provides sufficient reagents for 800 × 50-µl reactions.

## STORAGE CONDITIONS

**All Components:** –20°C

## ADDITIONAL MATERIALS REQUIRED

DNase-free $dH_20$
Temperature cycler
Thin-walled PCR tubes or PCR plates[||]
PCR primers
Deoxynucleotides (Agilent Catalog #200415)

[||] For the Agilent SureCycler 8800, use Agilent tube strips and caps (Catalog #410082 and #410086) or Agilent PCR plates (Catalog #401333 for 96-well plates and Catalog #410188 for 384-well plates). If using plates, seal the reactions with adhesive film (Catalog #410186) and a compression mat (Catalog #410187)

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# INTRODUCTION

Agilent SureStart *Taq* DNA polymerase is a modified version of *Taq2000* DNA polymerase with hot start capability. SureStart *Taq* DNA polymerase improves PCR amplification reactions by decreasing background and increasing amplification of desired products. Using SureStart *Taq*, hot start is easily incorporated into PCR protocols already optimized with *Taq* DNA polymerase, with little or no modification of cycling parameters or reaction conditions.

Preparing PCR reaction mixtures at room temperature can result in high background and lower yields of specific products. Certain PCR enzymes exhibit significant polymerase activity at temperatures encountered during reaction setup or while ramping up to stringent primer annealing temperatures. For example, *Taq* DNA polymerase exhibits 2–3% maximum activity at 25°C (e.g., room temperature setup) and 70% maximum activity at 50°C (which is generally below the melting temperature of PCR primers).[1] Nonspecific primer annealing and extension at nonrestrictive temperatures produces undesirable products that are amplified throughout the remaining cycles. Misprimed products and artifacts such as primer-dimers can impair gel analysis, quantitation, and sequencing of specific PCR products. In the amplification of misprimed products, dNTPs and primers are diverted away from the synthesis of specific products, reducing overall yields.

A number of hot start techniques have been developed to improve amplification specificity and to allow PCR setup at ambient temperatures.[2–6] The most convenient hot start methods employ reversibly inactivated enzymes. With SureStart *Taq* DNA polymerase, hot start is provided by a modified form of *Taq2000* DNA polymerase, a highly purified recombinant version of *Taq* DNA polymerase. SureStart *Taq* DNA polymerase remains inactive until stringent temperatures (e.g., 92–95°C) are reached. SureStart *Taq* can be activated by adding an initial step of 9–12 minutes at 92–95°C to the beginning of PCR cycling programs. Alternatively, the enzyme can be activated slowly during temperature cycling without prior activation, although it may be necessary to add additional cycles to existing cycling programs to achieve optimal product yield. Either activation method provides a PCR hot start, since primer extension can not occur during PCR setup when SureStart *Taq* DNA polymerase is inactive. SureStart *Taq* can be used in a variety of amplification systems, including quantitative PCR and RT-PCR, to improve specificity, yield, and amplification of difficult targets.

SureStart *Taq* DNA polymerase is optimized for use in amplifying DNA targets between 100 and 2000 bases. Although templates of up to 5 kb may be amplified with SureStart *Taq* DNA polymerase, we recommend the use of Agilent Herculase enhanced DNA polymerase to achieve optimal yields of longer targets (>2 kb).

# CRITICAL OPTIMIZATION PARAMETERS

SureStart *Taq* DNA polymerase can be introduced into all existing amplification systems optimized with *Taq* or *Taq2000* DNA polymerase, with only slight modification of cycling conditions. For other systems, optimization may be required to achieve highest product yield and specificity. Critical optimization parameters include the quality and concentration of DNA template and primers; the concentration of enzyme; cycling parameters; primer design; and the use of recommended reaction buffers.

## Primer–Template Purity and Concentration

The most successful PCR results are achieved when amplification reactions are performed using purified primers and templates that are essentially free of extraneous salts. Gel-purified primers, generally >18 nucleotides in length, are strongly recommended for use in SureStart *Taq*-based PCR.

Additionally, an adequate concentration of primers and template should be used to ensure a good yield of the desired PCR products. When DNA of known concentration is available, the reaction should contain at least $10^4$ DNA template molecules, but no more than 1 µg total template DNA. Amounts of 12.5–50 ng of DNA template/50-µl reaction are typically used for amplifying single-copy chromosomal targets. Amplifying a single-copy target from complex genomic DNA is generally more difficult than amplifying a fragment from a plasmid or phage. Less DNA template can be used for amplifying lambda or plasmid PCR targets or for amplifying multicopy chromosomal genes (typically 5–50 ng).[7] The mutation frequency can be reduced by limiting the number of PCR cycles; however, a corresponding increase in DNA template concentration is required to achieve comparable yields of PCR product. We recommend using primers at a final concentration of 0.1–0.5 µM, which is equivalent to ~50–125 ng of an 18- to 25-mer oligonucleotide primer in a 50-µl reaction volume.

## Enzyme Concentration

The concentration SureStart *Taq* DNA polymerase required for optimal PCR product yield and specificity depends on the individual target system to be amplified. Successful amplification can usually be achieved using 1.25 U of enzyme/50-µl reaction for PCR targets that are <2 kb. Further optimization will be required for targets greater than 2 kb.

## Cycling Parameters

As with all PCR reactions, cycling parameters are critical for successful amplification and may require further optimization.

### Denaturation Conditions

High denaturation temperatures can potentially damage DNA templates, so the denaturation temperature should be as low as possible. Denaturation temperatures of 92–95°C work well for most targets.

### Extension Time and Temperature

Use an extension temperature of 72°C and extension times of 0.5–1.0 minute for targets ≤ 1 kb and 1 min per kb for targets > 1 kb in length. Longer extension times may produce higher yields for difficult targets, such as high-complexity targets or targets > 2 kb in length.

## Activating SureStart *Taq*

Activation of the SureStart *Taq* DNA polymerase can be achieved by two methods: using a pre-PCR heat-activation method or by activating slowly over the course of the PCR amplification reaction (PCR heat-activation method).

### Using the Pre-PCR Heat-Activation Method

In this method, SureStart *Taq* DNA polymerase is partially or almost completely activated prior to temperature cycling. Activation is accomplished by adding an incubation step of 9–12 minutes at 92–95°C to the beginning of cycling programs. For certain amplification systems, the use of longer initial denaturation times (11–18 minutes) may improve product yield; however, background may also increase.

### Using the PCR Heat-Activation Method

For this method, the pre-PCR activation step is omitted and the enzyme is allowed to slowly activate during temperature cycling. For slow activation, we recommend using a standard initial denaturation step, e.g., 1 minute at 92–95°C. Due to lower initial DNA polymerase concentrations, product yield may be lower for some amplification systems after 30 cycles. In these cases, the use of additional cycles (5-10 cycles) is recommended to achieve optimal product yield.

The cycling conditions in Table II are recommended for use with the Pre-PCR Heat-Activation Method. When slow activation of SureStart *Taq* DNA polymerase is desired, the protocol can be modified as described in the footnotes of Table II.

## Primer Design

Primer pairs that exhibit similar melting temperatures and complete complementarity to the template are recommended. Primer sequences should be analyzed for potential duplex and hairpin formation as well as false priming sites in order to obtain the highest yield of specific PCR products. Depending on the primer design and the desired specificity of the PCR amplification reaction, melting temperatures between 55° and 80°C generally yield the best results.[7] The following formula[8] is commonly used for estimating the melting temperature ($T_m$) of the primers:

$$T_m(^\circ C) \cong 2(N_A + N_T) + 4(N_G + N_C)$$

where *N* equals the number of primer adenine (A), thymidine (T), guanidine (G), or cytosine (C) bases. Several other articles present additional equations for estimating the melting temperature of the primers.[9,10] Finally, care must be taken when using degenerate primers. Degenerate primers should be designed with the least degeneracy at the 3´ end. Optimization of degenerate primer concentration is necessary.

## Minimizing the Effects of Contaminating DNA

To minimize the possibility of DNA contamination, use dedicated pipettors with filter-tip pipet tips. Assemble PCR reactions in an area in the lab distinct from areas where PCR analysis is taking place. Dispensing buffer, sterile water, $MgCl_2$, primers, and dNTPs into small aliquots prior to use is prudent as small aliquots can be thrown away if they become contaminated.

## Reaction Buffer

For optimal yield and specificity it is essential that the recommended PCR buffer is used. Suboptimal results will be achieved using other buffers including the *Taq2000* DNA polymerase PCR buffer.

# POLYMERASE CHAIN REACTION PROTOCOL USING SURESTART *TAQ* DNA POLYMERASE

## Setting Up the PCR Reaction

1. Prepare a reaction mixture for the appropriate number of samples to be amplified. Add the components in order and mix gently. Table I provides an example of a reaction mixture for the amplification of a typical genomic or low complexity target. The recipe in Table I is for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl. Prior to setting up the reaction mixture, review *Critical Optimization Parameters* for guidelines.

**TABLE I**

**SureStart *Taq* DNA Polymerase Example Reaction Mixture**

| Component | Volume |
|---|---|
| $dH_20$, sterile | To 50 µl |
| SureStart *Taq* 10× reaction buffer | 5 µl |
| dNTP mix (25mM each dATP, dCTP, dGTP, dTTP) | 0.4 µl |
| Template DNA<br>Genomic (100 ng/µl)<br>OR<br>Low-complexity template (λ DNA or cloned DNA) (10 ng/µl) | <br>0.5 µl<br><br><br>0.5 µl |
| Primer #1 (100 ng/µl) | 1.25 µl |
| Primer #2 (100 ng/µl) | 1.25 µl |
| SureStart *Taq* DNA polymerase (5 U/µl) | 0.25 µl |

2. Immediately before thermal cycling, aliquot 50 µl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes.

3. Cycle the reactions according to the guidelines in Table II.

**TABLE II**

**Recommended Cycling Parameters[a,b]**

| Segment | Number of cycles | Temperature | Duration |
|---|---|---|---|
| 1 | 1 | 95°C | 9–12 minutes[c] |
| 2 | 30[d] | 95°C | 0.5–1 minute |
| | | Primer $T_m$ – 5°C[e] | 0.5–1 minute |
| | | 72°C | 0.5–1 minute for targets ≤ 1 kb<br>OR<br>1 minute /kb for targets > 1 kb |
| 3 | 1 | 72°C | 10 minutes |

[a] The recommended cycling conditions are for reactions carried out in thin-walled tubes (Agilent Catalog #410082 [tube strips] and #410086 [tube cap strips]).

[b] Cycling conditions may require modification depending on the thermal cycler used and the particular target being amplified. See cycling protocol recommendations provided by the thermal cycler manufacturer.

[c] For the pre-PCR heat-activation method, incubation for 9–12 minutes at the desired denaturation temperature (92–95°C) provides optimal results for most amplification systems. For the PCR heat-activation method, use an initial denaturation step of 1 minute at 92–95°C.

[d] For the PCR heat-activation method, cycle number can be increased to 35–40 cycles to achieve optimal product yield.

[e] The annealing temperature may be lowered or raised further if necessary to obtain optimal results. Typical annealing temperatures will range between 55 and 60°C.

## Examination of the PCR Products Using Gel Electrophoresis

Analyze the PCR amplification products by electrophoresis using an appropriate percentage acrylamide or agarose gel.

# TROUBLESHOOTING

| Observation | Suggestion |
|---|---|
| No PCR product or lower yield than expected | Ensure that template DNA is of sufficient quantity and quality (not sheared or nicked); increase template amount. |
| | If using the Agilent SureCycler 8800 to run PCR in plates sealed with film and a compression mat, decrease the denaturation time during cycling to 3–10 seconds whenever the reaction volume is <50 µl. |
| | Optimize the SureStart *Taq* DNA polymerase concentration; test increases in polymerase concentration of 0.25 units per 50 µl reaction. |
| | Optimize the $MgCl_2$ concentration by increasing in increments of 0.2 mM. |
| | For the pre-PCR heat-activation method, optimize the temperature or time used for the initial denaturation/activation step by increasing increments of 1°C or 1 minute, respectively. |
| | For the PCR heat-activation method, increase the total number of cycles to 35-45 cycles. For the pre-PCR heat-activation method, optimize cycle number by increasing in 3 cycle increments. |
| | Optimize annealing temperature by lowering in 2°C increments. |
| | Optimize annealing and extension times by increasing in 30 second increments. Allow at least 30 seconds of extension time for targets up to 1 kb, and increase extension times to 1 min per kb for targets > 1 kb. |
| | Ensure that the recommended 10× buffer supplied with SureStart *Taq* DNA polymerase was used. |
| | Confirm that primers are annealing or select alternate primer pairs for testing |
| | Use primer pairs with matched primer melting temps (Tm) and complete complementarity to the DNA template. |
| | Analyze the primers sequences to ensure that duplexes or hairpins do not form |
| | Gel-purified or HPLC purified primers ≥ 18 nucleotides in length are desired for successful PCR. |
| | Adjust ratio of primer vs. template to optimize yield. |
| | Use thin-walled tubes. |
| Product contains multiple bands | Optimize annealing temperatures by raising in 2°C increments. |
| | Use primer pairs with complete complementarity to target. |
| | Verify that the primers hybridize only to the desired sequences on the template. |
| | Analyze the primers to ensure that duplexes or hairpins do not form. |
| | Gel-purified or HPLC purified primers ≥ 18 nucleotides in length are desired for successful PCR. |
| | Select alternate primer pairs for testing. |
| | Try Agilent's Perfect Match PCR enhancer. |
| Product appears as a smear following agarose gel electrophoresis | Use clean pipettors, filter-tip pipet tips, and fresh solutions when setting up PCR reactions; work in an area in the lab distinct from areas where PCR analysis is taking place. |
| | Optimize SureStart *Taq* DNA polymerase concentration by decreasing in increments of 0.25 units per 50 µl reaction. |
| | Optimize denaturation time by increasing duration in increments of 30 seconds. |
| | Optimize denaturation temperature by increasing in 1°C increments |
| | Optimize extension time by reducing in 30 second increments |
| | Optimize cycle number by reducing in 3 cycle increments |

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## MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.