User Manual

Taq Full DNA Polymerase User Manual

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

PT3820-1 (PR4Z027)
Published 12 May 2005

Table of Contents

List of Figures

List of Tables

I. Introduction

Taq Full DNA Polymerase is a high-quality, full-length recombinant version of the Thermus aquaticus strain YT1 DNA Polymerase (Engelke et al., 1990). It is suitable for most general PCR amplification procedures. Taq Full DNA polymerase provides the best yield and greatest sensitivity of any Taq-derived polymerase. It can generate the highest yields from most DNA templates (including rare ones) ranging from bacterial and plasmid DNA to cDNA and complex genomic DNA.

The high efficiency, sensitivity, and robust nature of Taq Full enzyme translates into three primary advantages over other DNA polymerases:

— **Less template is needed**

Targets can be amplified using less template per reaction—specific amplification of a single-copy gene can be performed with as little as 1 ng of genomic DNA template (Figure 1). In situations where the amplification target is present at extremely low levels (e.g., amplifying a rare cDNA in an RT-PCR experiment or detecting viral nucleic acid), the heightened sensitivity of Taq Full DNA polymerase allows successful target gene amplification where other polymerases would fail.

— **Less enzyme is needed**

The high processivity and extension rate of Taq Full DNA polymerase enables you to use less enzyme in each PCR reaction. Favorable results can be generated using as little as 0.2 units in a single reaction (Figure 2).

— **Higher yields**

Taq Full Hot Start polymerase mix produces higher yields of each of the fragments in the 0.5–3.5 kb range than other hot start DNA polymerases (Figure 3). In addition, the Taq Full Hot Start enzyme consistently performs across this range of amplicon sizes. The efficiency of Taq Full Hot Start DNA polymerase and its ability to withstand a high number of PCR cycles allows you to obtain higher yields.

These advantages translate into significant benefits, whether your key objective is conserving limited and precious samples, or generating more data per unit of Taq Full DNA polymerase (or both).

Unlike other DNA polymerases currently offered by Clontech, Taq Full DNA polymerase is a recombinant version of the full-length, Thermus aquaticus enzyme (94 kD). (Our Advantage® , Advantage® 2, and TITANIUM™ PCR enzymes are truncated, N-terminal deletion mutants of wild-type Taq polymerase.) The Taq Full DNA polymerase has two catalytic activities: 1) It catalyzes the 5' to 3' polymerization of nucleotides into duplex DNA with a processivity of 50–60 nucleotides and an extension rate of about 75 nucleotides per second and 2) It contains a double-strand specific 5' to 3' exonuclease activity (reviewed in McPherson et al., 2000). Its half-life of 40 minutes at 95°C makes it highly suitable for lengthy PCR cycling conditions.

I. Introduction continued

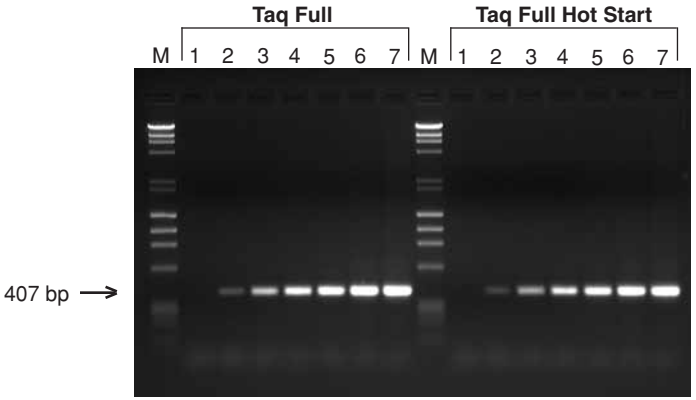

Figure 1. Amplification of a single-copy gene from calf thymus genomic DNA using Taq Full and Taq Full Hot Start DNA polymerase mixes. Specific amplification of the single-copy gene encoding bovine pancreatic trypsin inhibitor (BPTI) from various amounts of calf thymus genomic DNA template using 1 unit/reaction of Taq Full or Taq Full Hot Start DNA polymerase mixes. Reactions (50 µl) were performed using the following two-step cycling conditions: 94°C for 3 min; 30 cycles of 94°C for 30 sec, 68°C for 1 min; 68°C for 5 min. Lane 1: 0 ng template. Lane 2: 1 ng template. Lane 3: 5 ng template. Lane 4: 10 ng template. Lane 5: 20 ng template. Lane 6: 100 ng template. Lane 7: 200 ng template. M = Mixture (1:1) of λ DNA-Hind III and ΦX174-Hae III digests (NEB, Cat. Nos. N3012S & N3026S, respectively).

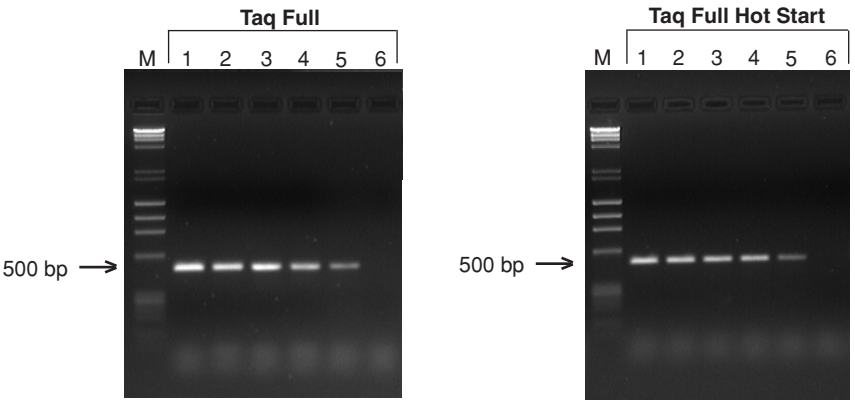

Figure 2. Less enzyme and template required per reaction with Taq Full and Taq Full Hot Start DNA polymerase mixes. Using a reduced amount of calf thymus genomic DNA template per reaction (10 ng), a 500 bp amplicon was amplified using decreasing numbers of units per reaction of Taq Full (Panel A) or Taq Full Hot Start (Panel B) DNA polymerase mix. Reactions were run under the following two-step cycling conditions: 94°C for 3 min; 30 cycles of 94°C for 30 sec, 68°C for 1 min; 68°C for 5 min. Lane 1: 5 units/reaction. Lane 2: 2.5 units/reaction. Lane 3: 1.0 units/reaction. Lane 4: 0.5 units/reaction. Lane 5: 0.2 units/reaction. Lane 6: 0 units/reaction. M = Mixture (1:1) of λ DNA-Hind III and ΦX174-Hae III digests (NEB, Cat. Nos. N3012S & N3026S, respectively).

I. Introduction continued

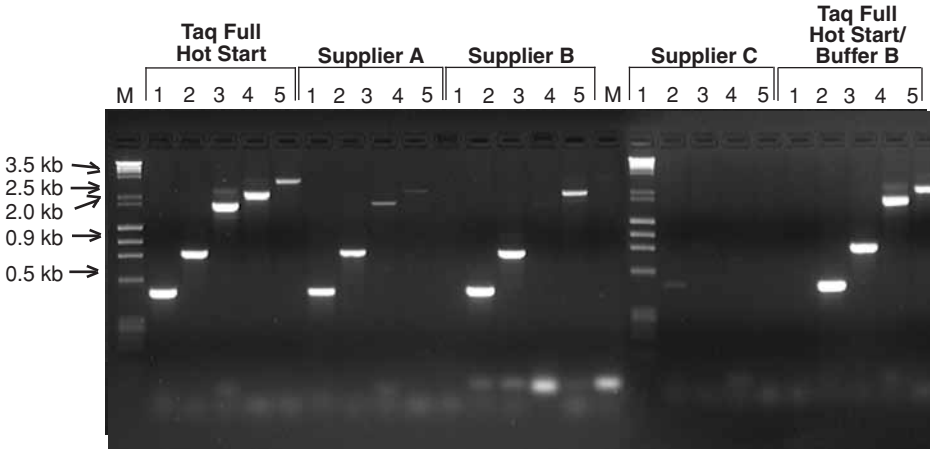

Figure 3. Taq Full Hot Start DNA polymerase mix provides higher yield and more consistent results than other hot start enzymes. Various-sized amplicons were produced from 100 ng of calf thymus genomic DNA template following manufacturer recommendations. Each reaction (50 µl) contained 2 units of enzyme, 1X PCR buffer with the appropriate amount of $MgCl_2$ for each enzyme, and 400 nM of each primer. Reactions were assembled at room temperature and performed using the following two-step cycling conditions: 94°C for 3 min; 30 cycles of 94°C for 30 sec, 68°C for 3 min 30 sec; 68°C for 5 min. Lane 1: 500 bp amplicon. Lane 2: 900 bp amplicon. Lane 3: 2,000 bp amplicon. Lane 4: 2,500 bp amplicon. Lane 5: 3,500 bp amplicon. M = Mixture (1:1) of λ DNA-Hind III and ΦX174-Hae III digests (NEB, Cat. Nos. N3012S & N3026S, respectively). The Taq Full Hot Start/Buffer B samples contain ammonium sulfate and 3 mM $MgCl_2$.

Automatic Hot Start with TaqStart® Antibody

The **Taq Full Hot Start DNA Polymerase Mix** (Cat. Nos. 639228, 639229 & 639230) and the **Taq Full Hot Start PCR Kit** (Cat. No. 639231) both contain built-in, hot start PCR. This is due to the presence of TaqStart® Antibody, which is conveniently included in the enzyme mix. TaqStart antibody is a highly efficient, high-affinity neutralizing monoclonal antibody that recognizes both full-length and N-terminal deletion versions of Taq. Antibody-mediated hot start with TaqStart Antibody has been shown to significantly improve the efficiency and specificity of PCR amplifications by reducing background DNA synthesis and increasing amplification of desired products (April 1994 Clontechniques; Kellogg et al., 1994). Certain PCR enzymes exhibit significant polymerase activity at temperatures encountered during reaction setup or while ramping. Inhibition of enzymatic activity at ambient temperatures ensures that our polymerase mix will result in greatly reduced background for most PCR applications by eliminating nonspecific primer annealing and extension products, and primer-dimer artifacts created prior to the onset of thermal cycling, both of which could contribute to amplification of undesirable products. Such mispriming reduces overall yield, impairs gel analysis and product quantitation, and may necessitate sequencing specific products to obtain useful information. With TaqStart antibody, more definitive results can be obtained when your target is either present in very low copy number or within a

I. Introduction continued

complex background of genomic DNA or cDNA. Full polymerase activity is then restored at the onset of thermal cycling because the antibody is denatured at high temperature during the first denaturation step.

Besides increased specificity and sensitivity, the built-in hot start using TaqStart antibody offers convenience. Other methods of hot start require extra steps such as the addition and premelting of wax beads, or the addition of a critical component after the initial denaturation. These extra steps are inconvenient, cumbersome, and introduce a potential source of cross-contamination. In contrast, because there is no need to add the antibody as a separate reagent during PCR setup, Taq Full Hot Start polymerase mix provides all the advantages of hot start PCR with none of the disadvantages of other current hot start methods.

Flexible and Convenient Formats

The Taq Full DNA Polymerase Enzyme is available in several formats to suit your application needs. It is available without TaqStart® Antibody in the **Taq Full DNA Polymerase Enzyme Mix** products (Cat. Nos. 639232, 639233, 639234 & 639235), and with TaqStart® Antibody in the **Taq Full Hot Start DNA Polymerase Enzyme Mix** products (Cat. Nos. 639228, 639229, 639230 & 639231). Addition of the TaqStart® Antibody provides automatic hot start PCR (Kellogg et al., 1994). Both enzymes are also available as enzyme mixes with PCR buffer (Cat. Nos. 639228, 639229, 639230, 639232, 639233 & 639234) or as convenient PCR Kits containing dNTP mixes, $MgCl_2$, and control primers and template in addition to the enzyme mix and buffer (Cat. Nos. 639231 & 639235).

No Buffer Optimization Needed

Taq Full DNA Polymerase allows you to easily perform PCR without tedious buffer optimization. Both the enzyme formulation and PCR kits are conveniently supplied with an optimized 10X Taq Full PCR Buffer (containing salts and a set concentration of 20 mM $MgCl_2$) suitable for most PCR applications. Additionally, both PCR kits come with a supplemental tube of 50 mM $MgCl_2$ to allow you to customize and titrate your reactions if required. In any given reaction, the Taq Full enzyme tolerates a wide range of Mg^{2+} concentrations. This formulation eliminates the need to add Mg^{2+} as a separate component during reaction setup. Our enzyme is also versatile–it performs just as well when used with an alternative PCR buffer containing ammonium sulfate and a higher $MgCl_2$ concentration (Figure 3).

I. Introduction continued

Recommended uses for Taq Full Products

Taq Full DNA polymerase mix is not intended for certain applications requiring very high fidelity PCR. In such cases we recommend Advantage® HF 2 PCR Kits (Cat. Nos. 639123 & 639124) specifically designed for long and accurate PCR. If you plan to amplify long (>5 kb) and/or highly complex templates for preparative purposes, we recommend our Advantage® 2 Mix and related kits (many). Additionally, the QTaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652 & 639655) is ideal for real-time qPCR applications and has been optimized for use with our wide selection of specialized Qzyme™ Assays (many). See the Related Products Section for ordering information.

II. List of Components

Store all components at –20°C.

Enough reagents are supplied for 100, 500, or 5,000 PCR reactions of 1 unit enzyme per 50 µl reaction volume.

Taq Full DNA Polymerase Enzyme Mix

Cat. No. 639232 (100 U)	Cat. No. 639233 (500 U)	Cat. No. 639234 (5,000 U)	
20 µl	100µl	1ml	**Taq Full DNA Polymerase Mix** Includes Taq Full DNA Polymerase (5 units/µl) in storage buffer (20 mM Tris-HCl [pH 8.0], 100 mM KCl, 0.1 mM EDTA, 1.0 mg/ml BSA, and 50% Glycerol).
1.25 ml	3x1.25 ml	30 ml	**10X Taq Full PCR Buffer** Includes 200 mM Tris-HCl (pH 8.5), 500 mM KCl, 20 mM $MgCl_2$, and 0.1% Tween 20.

Taq Full Hot Start DNA Polymerase Enzyme Mix

Cat. No. 639228 (100 U)	Cat. No. 639229 (500 U)	Cat. No. 639230 (5,000 U)	
20 µl	100 µl	1 ml	**Taq Full Hot Start DNA Polymerase Mix** Includes Taq Full DNA Polymerase (5 units/µl) and TaqStart® Antibody (1.1 µg/µl) in storage buffer (15 mM Tris-HCl [pH 8.0], 75 mM KCl, 0.05 mM EDTA, 0.5 mg/ml BSA, and 50% Glycerol).
1.25 ml	3x1.25 ml	30 ml	**10X Taq Full PCR Buffer** Includes 200 mM Tris-HCl (pH 8.5), 500 mM KCl, 20 mM $MgCl_2$, and 0.1% Tween 20.

II. List of Components continued

Taq Full DNA Polymerase PCR Kit (Cat. No. 639235)

- **20 µl Taq Full DNA Polymerase Mix** (5 units/µl)
  Includes Taq Full DNA Polymerase (5 units/µl) in storage buffer (20 mM Tris-HCl [pH 8.0], 100 mM KCl, 0.1 mM EDTA, 1.0 mg/ml BSA, and 50% Glycerol).
- **1.25 ml 10X Taq Full PCR Buffer** (200 mM Tris-HCl [pH 8.5], 500 mM KCl, 20 mM $MgCl_2$, and 0.1 % Tween 20)
- **100 µl dNTP Mix** (10 mM each of dATP, dCTP, dGTP, and dTTP)
- **500 µl $MgCl_2$** (50 mM)
- **10 µl Control Genomic DNA Template** (Calf Thymus DNA;100 ng/µl)
- **20 µl Control Primer Mix** (407 bp bovine pancreatic trypsin inhibitor (BPTI) gene forward and reverse primers; 10 µM each)

  5' primer 5'–CTTGTCCTAATCTTCCTCCTCACGGCA–3'

  3' primer 5'–TGGCACGGCCATAAGAGGTAGATGTCA–3'
- **4x1.25 ml PCR-Grade Water**

Taq Full Hot Start DNA Polymerase PCR Kit (Cat. No. 639231)

- **20 µl Taq Full Hot Start DNA Polymerase Mix** (5 units/µl)
  Includes Taq Full DNA Polymerase (5 units/µl) and TaqStart® Antibody (1.1 µg/µl) in storage buffer (15 mM Tris-HCl [pH 8.0], 75 mM KCl, 0.05 mM EDTA, 0.5 mg/ml BSA, and 50% Glycerol).
- **1.25 ml 10X Taq Full PCR Buffer** (200 mM Tris-HCl [pH 8.5], 500 mM KCl, 20 mM $MgCl_2$, and 0.1 % Tween 20)
- **100 µl dNTP Mix** (10 mM each of dATP, dCTP, dGTP, and dTTP)
- **500 µl $MgCl_2$** (50 mM)
- **10 µl Control Genomic DNA Template** (Calf Thymus DNA;100 ng/µl)
- **20 µl Control Primer Mix** (407 bp bovine pancreatic trypsin inhibitor (BPTI) gene forward and reverse primers; 10 µM each)

  5' primer 5'–CTTGTCCTAATCTTCCTCCTCACGGCA–3'

  3' primer 5'–TGGCACGGCCATAAGAGGTAGATGTCA–3'
- **4x1.25 ml PCR-Grade Water**

III. Additional Materials Required

The following reagents and equipment are required but not supplied:

- **Thermal cycler** (heated lid or non-heated lid)
- **Pipettors** dedicated for pre-PCR (no template) work
- **Aerosol-free PCR pipette tips** suitable for use with the above pipettors and preferably equipped with hydrophobic filters.
- **Gel electrophoresis equipment**
- **DNA size markers**
- **5X Stop/Loading buffer**
  (Sambrook et al. [2001] provides several recipes)
- **0.5 ml PCR reaction tubes or multi-well plates**

  (We recommend Applied Biosystems GeneAmp PCR Reaction Tubes [0.5 ml; Cat. No. N801-0737], GeneAmp PCR Reaction Tubes with Flat Cap [0.5 ml; Cat. No. N801-0180] or MicroAmp Optical 96-well Reaction Plates [Cat. No. N801-0560].)
- [Optional] **Mineral oil** for overlaying samples run on a non-heated lid thermal cycler (We recommend Sigma Cat. No. M3516.)

The following reagents are supplied with the **Taq Full DNA Polymerase PCR Kits** (Cat. Nos. 639235 & 639231) but not with the **Taq Full DNA Polymerase Enzyme Mixes** (Cat. Nos. 639228, 639229, 63930, 639232, 639233 & 639234):

- **Advantage Ultrapure PCR Deoxynucleotide Mix** (Cat. No. 639125)
- **Positive Control Genomic DNA Template and Control Primer Mix** (Forward and reverse primers; 10 µM each)
- **50 mM $MgCl_2$** (Allows you to customize your reactions if a final $MgCl_2$ concentration greater than 2 mM is required.)
- **PCR-Grade Water**
  Avoid using autoclaved H_2O because the recycled steam in some autoclaves can introduce contaminants that may interfere with PCR.

IV. General Considerations

A. Primer Design

Primer design is the single largest variable in PCR applications and the single most important factor in determining the success or failure of PCR reactions. **Always check and recheck your primer design before constructing or ordering primers**. For helpful guidelines on primer design, please visit the following web site:

http://www.basic.nwu.edu/biotools/oligocalc.html

The Taq Full system can be used in a wide variety of PCR applications, and the constraints on primer design will vary from one application to the next. The following general rules are provided as guidelines.

T_m: Design primer pairs with similar T_m values. For most applications, we prefer two-step cycles (denaturation at T_1 followed by annealing and extension at T_2) instead of three-step cycles (denaturation at T_1 followed by annealing at T_2 followed by extension at T_3). Three-step cycles will be necessary when the T_m of the primers is less than 60–65°C and in certain special protocols. See Table II for cycling parameters.

Annealing temperature: Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature of [T_m – 5°C].

Length: Primers should be **at least** 22 nucleotides (nt) long. 25–30-mers are preferred.

G-C content: 45–60%. Avoid runs of 3 or more Gs or Cs at 3' ends

Complementary sequences: Avoid complementary sequences within the entire primer sequence as well as between primer pairs. This is especially crucial for the terminal 2–3 bases at the 3' ends of primer pairs in order to avoid primer-dimer formation.

3' end: Avoid using a T residue as this increases the chance of a mismatch.

B. Template Purity and Quantity

Both the purity and quantity of the starting DNA template significantly affect PCR sensitivity and efficiency. Because PCR amplification proceeds exponentially, many conventional PCR applications work well with templates of average or even low quality. In many applications, Taq Full DNA polymerase will tolerate a wide range of template quality. Template quality becomes much more important when amplifying from longer, more complex targets, or when desiring the highest possible sensitivity. Many methods are available to optimally purify your template DNA. NucleoSpin® columns (many) yield rapid minipreparations of plasmid or genomic DNA using centrifugation and/or vacuum manifolds. For large-scale preparations that require no centrifugation, NucleoBond® columns (many) may be used. The optimal nucleic acid purification product depends on your starting material, DNA amount being purified, and end application(s). These products

IV. General Considerations continued

are available in a wide array of formats and sizes for DNA purification of various DNA types (plasmid, genomic, BAC, Lambda, plant, and viral) and sources (cell cultures, tissues, and blood). Our NucleoSpin® Extract Kits (many) allow extraction of DNA fragments from PCR reactions while the NucleoFast (many) and NucleoTrap PCR Purification Kits (Cat. Nos. 636054 & 636020) purify away unwanted PCR components (primer-dimers, high salts, dNTPs, and PCR primers). Please visit our web site at **www.clontech.com** for detailed information regarding which nucleic acid or PCR purification products best suit your needs.

C. Amplicon Size

Taq Full enzyme has successfully amplified targets of up to 3.5 kb in size from genomic DNA (Figure 3).

D. Background Amplification

Due to the sensitivity and robustness of the Taq Full enzyme, nonspecific background amplification may sometimes result. Try using our Taq Full Hot Start polymerase mix if you are not already doing so. Additionally, you may need to adjust the cycle number or annealing temperature. See Section VI.B for more troubleshooting suggestions.

E. Good PCR Practices

1. Use a dedicated space and dedicated equipment

   Prepare reactions in a PCR workstation using dedicated pipettors in a dedicated work space that is free of contaminating DNA. Due to the tremendous amplification power of PCR, minute amounts of contaminating DNA can produce nonspecific amplification. We recommend that you use small aliquots of starting material to avoid contaminating your stocks. As with all PCR experiments, always wear gloves, use PCR pipette tips with hydrophobic filters and dedicated solutions, and perform post-PCR analysis in a separate area. We also recommend setting up a negative control reaction that lacks template.

2. Practice careful pipetting

   Because of the small volumes used in PCR experiments, careful pipetting technique is extremely important. Always verify that no extra solution is carried over on the outside of the pipette tip before transfer. When adding liquid to a tube, immerse the tip into the reaction mixture, deliver the contents from the pipette tip into the mixture, and then rinse the tip by pipetting up and down several times.

3. Use a Master Mix

   Assembling a Master Mix, which contains the appropriate volumes of all reagents required for multiple reactions, eliminates repeated pipetting of individual reaction components into each reaction tube. In addition, it greatly reduces sample-to-sample variation.

IV. General Considerations continued

4. Always include positive and negative controls (i.e., PCR-grade H_2O instead of DNA template).

F. Touchdown PCR

"Touchdown" PCR can significantly improve the specificity of many amplfication reactions in a wide variety of applications by increasing the abundance of the initial primer-template duplex (Don et al., 1991; Roux, 1995). Briefly, touchdown PCR involves using an annealing/extension temperature that is several degrees **higher** (typically 3–10°C) than the T_m of the primers during the initial phase (typically 5–10 cycles). In subsequent cycles, the annealing temperature is decreased in 1–2°C increments per cycle until a temperature is reached that is equal to, or 2–5°C below, the T_m of the primers. This change can be performed either in a single step or in increments over several cycles.

G. Use of additives

TaqStart Antibody binds Taq Full DNA polymerase with high affinity under the conditions described in this User Manual. The addition of 2–5% DMSO will not interfere with antibody function and may improve results in some instances. However, the addition of formamide or other cosolvents may disrupt antibody function. Furthermore, excessive amounts of glycerol, solutes (e.g., salts), pH extremes, and/or other deviations from the recommended reaction conditions may reduce the effectiveness of both TaqStart antibody and Taq Full DNA polymerase.

V. Taq Full PCR Procedure

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

IMPORTANT CONSIDERATIONS

For best results, all components should be fully thawed, vortexed briefly, and then stored on ice until needed.

If using Taq Full DNA Polymerase without Hot Start, optimal results are obtained when all components and reactions, including Master Mix , are assembled and maintained on ice. Preparation at room temperature may result in higher background and lower yield of specific products. These set-up conditions are not as crucial for reactions containing TaqStart Antibody (i.e., Taq Full Hot Start products).

A. Control PCR Reactions

Positive and negative control PCR reactions should be performed in parallel with your experiments to ensure that the Taq Full DNA Polymerase Mix is working properly.

In addition to enzyme and 10X Buffer, our Taq Full (Cat. No. 639235) and Taq Full Hot Start (Cat. No. 639231) PCR Kits include Positive Control Genomic DNA template and Control Primer Mix, as well as dNTP Mix and PCR-Grade Water. For all other Taq Full Enzyme Mix products (Cat. Nos. 639228, 639229, 639230, 639232, 639233 & 639234), you must supply these components separately and therefore you may need to adjust the actual component volumes from the values found in Table I.

1. Set up the **Control Reactions** (Table I).

TABLE I. CONTROL REACTION MIXES (50 µl)

Reagent	Positive	Negative	Final Concentration
10X Taq Full PCR Buffer (containing 20 mM $MgCl_2$)	5 µl	5 µl	1X (2 mM final)
dNTP Mix (10 mM each)	1 µl	1 µl	200 µM each
Control Primer Mix (10 µM each)	2 µl	2 µl	0.4 µM
Taq Full DNA Polymerase Mix	0.2 µl	0.2 µl	1 unit
Control Genomic DNA Template (100 ng/µl)	1 µl	–	100 ng
PCR-Grade Water	up to 50 µl	up to 50 µl	

2. Mix well and spin tube briefly.

   Note: When using a heated lid thermal cycler, do not overlay samples with mineral oil as this may decrease overall product yield.

V. Taq Full PCR Procedure continued

3. Start thermal cycling using the following parameters:
   - **Initial denaturation**

     94°C for 3 min[a]
   - **PCR cycles** (30 cycles)[b]

     Denaturation: 94°C for 30 sec
     Annealing/Extension: 68°C for 1 min
   - **Additional Extension**

     68°C for 5 min

   [a] Use the minimal possible time. In some cases, better results may be obtained by modifying the denaturation step (e.g., 94°C for 15 sec). Exposure of DNA to high temperatures causes some depurination of single-stranded DNA during denaturation, which eventually leads to strand scission. High temperature also leads to gradual loss of enzyme activity. **Important:** If you are using Taq Full DNA polymerase without hot start antibody, the initial denaturation cycle applies only to DNA template denaturation; thus, the duration of the initial denaturation can be reduced to 1 min.

   [b] 30–35 cycles for single- or low-copy-number genes or rare cDNAs. For most applications, we prefer two-step cycles (denaturation at T_1 followed by annealing and extension at T_2) instead of three-step cycles (denaturation at T_1 followed by annealing at T_2 followed by extension at T_3). Three-step cycles will be necessary when the T_m of the primers is less than 60–65°C and in certain special protocols.

   Note: The supplied Control Template and Control Primer Mix have been optimized with two-step cycling parameters, but are also suitable for use with a three-step program. See Table II below for examples of suitable cycling parameters.

4. Store reactions at 4°C or –20°C until needed.
5. Analyze 5 µl of your PCR reaction by electrophoresis on a 0.8–1.5% agarose/EtBr gel. See Section V.C for gel electrophoresis details.

Expected results: Using the positive Control Template and Control Primer Mix supplied in the kit, the reaction should produce a single major band of 407 bp from the bovine pancreatic trypsin inhibitor gene (See Figure 1). No bands should be generated in the negative control (no DNA template).

TABLE II. EXAMPLES OF TYPICAL CYCLING PARAMETERS

Two-step program	Three-step program
• 94°C for 3 min	• 94°C for 3 min
• 30 cycles: 94°C for 30 sec 68°C for 1 min	• 30 cycles: 94°C for 30 sec 68°C for 30 sec 72°C for 1 min
• 68°C for 5 min	• 72°C for 5 min

V. Taq Full PCR Procedure continued

B. Recommended Guidelines for Taq Full Test PCR Reactions

Use the following guidelines when setting up your initial experiments with Taq Full DNA polymerase mix. These are general guidelines—optimal parameters may vary with different thermal cyclers and will depend on your particular primers and template, as well as other experimental variables.

1. Preparation of Master Mix

   Whenever possible, use a Master Mix for multiple reactions of the same type. The Master Mix contains all components required as listed below, **except** the DNA template, which is added last in Step 1.c. To ensure that you have sufficient Master Mix, prepare enough for all reactions plus one additional reaction.

   a. Combine the following reagents in an appropriately sized PCR tube (Table III).

   Note: If using Taq Full DNA polymerase without Hot Start, optimal results are obtained when all components and reactions, including the Master Mix, are assembled and maintained on ice. Preparation at room temperature may result in higher background and lower yield of specific products. These set-up conditions are not as crucial for reactions containing TaqStart antibody.

TABLE III. MASTER MIX PREPARATION

Reagent	Amount per reaction	Final
10X Taq Full PCR Buffer (containing 20 mM $MgCl_2$)[a]	5 µl	1X (2 mM)
dNTP Mix (10 mM each)	1 µl	200 µM each
Primer mix (10 µM each)	2 µl	0.4 µM
Taq Full DNA Polymerase Mix	0.2–0.5 µl	1–2.5 units[b]
Template DNA	1–5 µl	variable
PCR-Grade Water	up to 50 µl	–

[a] Additional 50 mM $MgCl_2$ is provided in the PCR Kits to allow you to establish your reactions with a final $MgCl_2$ concentration higher than 2 mM if necessary.

[b] A range of 1–2.5 unit per reaction is appropriate for most applications. Longer amplicons and more complex templates may require more units per reaction, but final titrations should be empirically determined for each application.

   b. Mix well and spin briefly.

   c. Add DNA template.

   Note: When using a heated lid thermal cycler, do not overlay samples with mineral oil as this may decrease overall product yield.

V. Taq Full PCR Procedure continued

2. General cycling parameter guidelines

   Use the guidelines in Step A.3 and Table II when setting up your initial experiments with the Taq Full system. These are general guidelines—the optimal parameters may vary with different thermal cyclers and will depend on your particular primers, template, and other experimental variables. See the Troubleshooting section to address particular issues associated with determining PCR cycling parameters.

C. Recommendations for electrophoresis

We recommend that you transfer a 5 µl sample of your PCR reaction to a fresh tube and add 1 µl of 5X Stop/Loading buffer. Place the remaining 45 µl of the reaction mixture on ice; it can be subjected to further cycling if you do not see a product. Analyze your sample(s), along with suitable DNA size markers, by electrophoresis on an appropriate agarose gel containing 0.1 µg/ml EtBr. The percentage of agarose and the DNA size markers you choose will depend on your expected range of product sizes. You may find the following general guidelines helpful in choosing your gel:

Recommendations for agarose gels:

Expected insert size range	Recommended % agarose	Recommended DNA size markers
0.3–1.5 kb	1.5	ϕX174/Hae III
0.5–10 kb	1.2	1 kb DNA ladder
>5 kb	0.8	λ/Hind III

VI. Troubleshooting Guide

The following **general** guidelines apply to most PCR reactions. However, this User Manual does not comprehensively address troubleshooting guidelines for all of the applications for which Taq Full products can be used. When using Taq Full DNA polymerase with a companion or accessory Clontech product, please refer to the appropriate, application-specific User Manual.

A. No product observed or low yield

PCR component missing/degraded	Use a checklist when assembling reactions. Always perform a positive control to ensure that each component is functional. If the positive control does not work, repeat the positive control only. If the positive control still does not work, repeat again, replacing individual components to identify the faulty reagent.
Cycle number inadequate	Increase the number of cycles by 5 additional cycles at a time, up to 40 cycles.
Annealing temp. too high	Decrease the annealing temperature in increments of 2–4°C.
Primer concentration not optimal	Perform titration of primer concentration in multiple reactions.
Primer design not optimal	Redesign your primer(s) after confirming the accuracy of the sequence information. If the original primer(s) was less than 22 nt long, try using a longer primer. If the original primer(s) had a G-C content of less than 45%, try to design a primer with a G-C content of 45–60%.
Template amount insufficient	Repeat PCR using a higher concentration of DNA (after amplification using more cycles).
Template quality poor	Check template integrity by electrophoresis on a standard TBE-agarose gel. If necessary, repurify your template using methods that minimize shearing and nicking, and never vortex your template or freeze-thaw multiple times.
Extension time inappropriate	Especially when working with longer templates, increase extension time in 1 min increments. We recommend 1 min extension per kb of template.

VI. Troubleshooting Guide continued

Enzyme concentration not optimal	The Taq Full DNA Polymerase Enzyme Mix can be used at 1 unit (per 50 µl reaction) for most routine applications but can be increased up to 2.5 units per 50 µl reaction.
[Mg^{2+}] not optimal	The Taq Full DNA Polymerase Enzyme Mix performs well for most applications using a 2 mM Mg^{2+} concentration (broad range of amplicon sizes and template types). Therefore, as long as you use the buffer included with the polymerase mix and a final concentration of 0.2 mM of each dNTP, it is unlikely that a lack of product is due to problems with the Mg^{2+} concentration. However, high concentrations of EDTA or other metal chelators in the template stock solution can reduce the effective concentration of Mg^{2+} to a suboptimal level. In those cases, perform PCR reactions using increasing amounts of Mg^{2+} from 2.0–3.5 mM (final concentration in a 50 µl reaction).
[dNTPs] not optimal	When used as recommended, the dNTP Mix provided with the kit gives a final concentration of 0.2 mM of each dNTP. In our experience, this concen-tration of dNTPs is suitable for a wide range of applications. If you are preparing your own dNTPs, be sure that your final concentration of each dNTP in the reaction is 0.2 mM. We recommend using either our Advantage Ultrapure PCR Deoxynucleotide Mix (Cat. No. 639125) or dNTP Combination Kit (Cat. No. 639132). Note that if you increase the concentration of dNTPs, you will also need to increase the [Mg^{2+}] proportionately.
Target difficult to amplify	Some targets are inherently difficult to amplify. If you are amplifying a complex template, a template with unusually high G-C content, or a low abundance gene, you may need to increase the amount of starting template. Advantage GC 2 Polymerase Mix and PCR Kits (Cat. Nos. 639114,639120 & 639119) are recommended in cases of unusually high G-C content and/or secondary structure.

VI. Troubleshooting Guide continued

B. Multiple products

Hot start required	If you choose to use Taq Full DNA Polymerase without Hot Start, you will need to assemble your reactions on ice. You may also want to use a conventional hot start method such as adding enzyme only after the reaction has been heated.
Cycle number inappropriate	Reducing the cycle number may eliminate nonspecific bands. Try Touchdown PCR (see Section IV.F).
Annealing temperature too low	Increase the annealing/extension temperature in increments of 2–3°C.
Primer design not optimal	See Section A.
Touchdown PCR required	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications (Don et al., 1991; Roux, 1995). Touchdown PCR uses an annealing/extension temperature that is several degrees **higher** than the T_m of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer T_m for the remaining PCR cycles. The change can be performed either in a single step or in increments over several cycles.
Contamination	See Section E.
Less enzyme required	If smearing is observed, first try optimizing the cycle parameters as described above, then try reducing the enzyme concentration.
Less template required	Try a lower concentration of DNA template in the PCR reaction.

C. Low yield

Template quality poor	Check template integrity by electrophoresis on a standard TBE-agarose gel. If necessary, repurify your template using methods that minimize shearing and nicking.

D. Products are smeared on gel

Cycle number inappropriate	Reduce the cycle number by 3–5 cycles to see if nonspecific bands are eliminated.

VI. Troubleshooting Guide continued

Denaturation temperature too low	Try increasing the denaturation temperature in increments of 1°C.
Extension time inappropriate	Decrease the extension time in 1–2 min increments.
Primer concentration not optimal	Perform titration of primer concentration in reactions.
Poor template quality	See Section A.
Touchdown PCR required	See Section IV.F.
Hot start required	If you choose to use Taq Full DNA Polymerase without TaqStart Antibody, you will need to assemble your reactions on ice. You may want to use a conventional method of hot start such as adding enzyme only after the reaction has been heated.

E. Dealing with contamination

Contamination most often results in extra bands or smearing. It is important to include a no-template negative control (a control that replaces the DNA template with PCR-grade H_2O but still includes the primers) in every PCR experiment to determine if the PCR reagents, pipettors, or PCR reaction tubes are contaminated with previously amplified targets.

If possible, set up separate, dedicated laboratory areas (with separate sets of pipettors) for your pre-PCR reaction set-ups and post-PCR analyses.

Laboratory benches and pipettor shafts can be decontaminated by depurination. Wipe surfaces with 1N HCl followed by 1N NaOH. Then neutralize with a neutral buffer (e.g., Tris or PBS) and rinse with ddH_2O.

We highly recommend using commercially available aerosol-free pipette tips for all liquid manipulations.

An enzymatic method has been published for destroying PCR product carryover (Longo et al., 1990). It involves incorporation of dUTP into the PCR products and subsequent hydrolysis with uracil-N-glycosylase (UNG).

When performing PCR directly on phage plaques or bacterial colonies, failure to isolate single plaques or colonies will also produce multiple bands.

VII. References

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K. & Mattick, J. S. (1991) "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. **19**:4008.

Engelke, D. R., Krikos, A., Bruck, M. E. & Ginsburg, D. (1990) Purification of Thermus aquaticus DNA polymerase expressed in Escherichia coli. Analytical Biochemistry **191**: 396-400.

Kellogg, D. E., Rybalkin, I., Chen, S., Mukhamedova, N., Vlasik, T., Siebert, P. & Chenchik, A. (1994) TaqStart Antibody: Hot Start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. BioTechniques **16**:1134–1137.

Kim, Y., Eom, S. H., Wang, J., Lee, D. S., Suh, S. W. & Steitz, T. A. (1995) Crystal structure ofThermus aquaticus DNA polymerase. Nature **376**:612–616.

Lawyer, F. C., Stoffel, S., Saiki, R. K., Myambo, K., Drummond, R. & Gelfand, D. H. (1989) Isolation, characterization, and expression in Escherichia coli of the polymerase gene from Thermus aquaticus. J. Biol. Chem. **264**: 6427-6437.

Lawyer, F. C., Stoffel, S., Saiki, R. K., Chang, S-Y., Landre, P. A., Abramson, R. D. & Gelfand, D. H. (1993) High-level expression, purification, and enzymatic characterization of full-lengthThermus aquaticus DNA Polymerase and a truncated form deficient in 5' to 3' exonuclease activity.PCR Methods and Applications. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Longo, M. C., Berninger, M. S. & Hartley, J. L. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene **93**:3749.

McPherson., M. J. & Møller, S., G. (2000) PCR: The Basics (from background to bench). (Springer-Verlag Telos, New York, NY).

Roux, K. H. (1995) Optimization and troubleshooting in PCR. PCR Methods Appl. **4:**5185–5194.

Sambrook, J., Russell, D. W., & Sambrook, J. (2001) Molecular Cloning: A Laboratory Manual, Third Edition (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

TaqStart®:A neutralizing monoclonal antibody that facilitates "Hot Start" PCR (April 1994) Clontechniques **IX**(2):1–5.

VIII. Related Products

For a complete listing of all Clontech products, please visit **www.clontech.com**

Products	Cat. No.
• TaqStart® Antibody	639250 639251
• Advantage® HF 2 PCR Kit	639124 639123
• QUICK-Clone™ cDNAs	many
• Marathon cDNA Amplification Kit	634913
• Marathon-Ready cDNAs	many
• Sprint™ TITANIUM™ Taq 384 Plate	639552
• Sprint™ Advantage® 96 Plate	639550
• Sprint™ Advantage® Single Shots	639556 639553 639554
• TITANIUM™ Taq DNA Polymerase	639208 639209
• TITANIUM™ Taq PCR Kit	639211 639210
• TITANIUM™ One-Step RT-PCR Kits	639503 639504
• SMART™ cDNA Library Construction Kit	634901
• Advantage® 2 Polymerase Kit	639201 639202
• Advantage® 2 PCR Kit	639206 639207
• 10X Advantage® 2 PCR Buffer	639137 639138
• 10X Advantage® 2 SA PCR Buffer	639147 639148
• Advantage® Genomic Polymerase Mix	639110
• Advantage® Genomic PCR Kit	639104 639103
• Advantage® GC Genomic Polymerase Mix	639113
• Advantage® GC Genomic PCR Kit	639118 639117

VIII. Related Products continued

Products	Cat. No.
• Advantage® GC 2 Polymerase Mix	639114
• Advantage® GC 2 PCR Kit	639120 639119
• Advantage® UltraPure PCR Deoxynucleotide Mix	639125
• Advantage® UltraPure dNTP Combination Kit 639132	
• Advantage® RT-for-PCR Kits	639505 639506
• Super SMART™ PCR cDNA Synthesis Kit	635000
• SMART™ RACE cDNA Amplification Kit	634914
• Human GenomeWalker™ Kit	638901
• Mouse GenomeWalker™ Kit	638902
• Rat GenomeWalker™ Kit	638903
• GenomeWalker™ Universal Kit	638904
• PCR-Select™ cDNA Subtraction Kit	637401
• PCR-Select™ Bacterial Genome Subtraction Kit	637404
• LD-Insert Screening Amplimer Sets	many
• RT-PCR Control Amplimer Sets	many
• MTC™ Multiple Tissue cDNA Panels	many
• NucleoSpin® Kits	many
• NucleoSpin® Extract Kits	many
• NucleoBond® Kits	many
• NucleoFast Kits	many
• NucleoTrap PCR Purification Kits	636054 636020

Notes

Notes

Notes

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes, nor is it intended for human use. Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

Advantage® 2 products are covered by U.S. Patent No. 5,436,149.

SMART™ Technology is covered by U.S. Patents Nos. 5,962,271 & 5,962,272. For-Profit and Not-For-Profit purchasers of SMART™ Products are entitled to use the reagents for internal research. However, the following uses are expressly prohibited: (1) performing services for third parties; (2) identifying nucleic acid sequences to be included on nucleic acid arrays, blots, or in libraries or other cDNA collections which are then sold to third parties. Reproduction, modification, reformulation, or resale of the reagents provided in SMART™ Products is not permitted. For information on licensing SMART™ Technology for commercial purposes, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

TaqStart® Antibody is licensed under U.S. Patent No. 5,338,671.

Clontech® PCR-Select™ cDNA Subtraction products are covered by U.S. Patent Nos. 5,565,340 and 5,759,822, as well as pending foreign patent applications. For-Profit and Not-For-Profit purchasers of PCR-Select products are entitled to use the reagents for internal research, including identification of molecular markers or differentially expressed genes; however, the following uses are expressly prohibited: (1) performing services for third parties; (2) identifying nucleic acid sequences to be included on nucleic acid arrays, blots, or in libraries or other cDNA collections which are then sold to third parties; or (3) constructing databases which are then sold to third parties. Reproduction, amplification, modification, reformulation, or resale of the reagents provided in PCR-Select products is not permitted. For information on licensing PCR-Select for commercial purposes, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

MicroAmp® is a registered trademark of Applera Corporation or its subsidiaries in the US and certain other countries.

NucleoSpin® and NucleoBond® are registered trademarks of MACHEREY-NAGEL GmbH and Co. KG.

Tween20® is a registered trademark of of ICI Americas, Inc.