User Manual

TaqStart™ Antibody 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

Cat. Nos. 639250
639251
639252
PT1576-1 (PR52153)
Published 2 March 2005

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I. Introduction

Hot start PCR is commonly used to enhance the specificity and sensitivity of PCR (D'Aquila *et al.*,1991; Chou *et al.*, 1992; Faloona *et al.*, 1990). In many cases, hot start PCR produces greater yields of single products than has been possible with conventional PCR. However, manual hot start PCR is inconvenient, time-consuming, and incurs a risk of cross-contamination. TaqStart™ Antibody makes it possible to perform TaqStart PCR, with all the advantages—and none of the disadvantages—of hot start PCR.

T aqStart Antibody is a neutralizing monoclonal antibody to *Taq* DNA polymerase (Kellogg *et al.*, 1994; Findlay *et al.*, 1993; Sharkey *et al.*, 1994). TaqStart Antibody is used to block polymerase activity during setup of the PCR reactions at ambient temperatures (20–22°C). The inhibition of *Taq* DNA polymerase is completely reversed when the temperature is raised above 70°C. At the first template denaturation step in thermal cycling, the enzyme-antibody complex dissociates and the TaqStart Antibody is rendered nonfunctional. At the same time, the activity of *Taq* DNA polymerase is restored, and the enzyme functions normally during the course of the PCR. TaqStart Antibody is effective with a variety of commercially available *Taq* DNA polymerases (native and recombinant). TITANIUM™ *Taq* products and all of our Advantage™ 2 products are formulated with TaqStart Antibody.

The use of TaqStart Antibody significantly improves the results of PCR amplifications that can be enhanced by a hot start. In many cases, TaqStart PCR has been proven to prevent generation of nonspecific amplification products and primer-dimer artifacts. This means that more definitive PCR results can often be obtained in cases where generation of nonspecific amplification products is a problem. Typical applications for TaqStart Antibody include PCR reactions involving one or more of the following: complex genomic DNA or cDNA templates; very low-copy-number targets; large numbers of thermal cycles (>35); and multiple primer pairs in the same tube (i.e., multiplex PCR).

II. List of Components

The amounts provided are sufficient for 200 or 500 reactions (using 2 units *Taq* DNA polymerase per reaction). Store components at –20°C.

Cat. No. 639250 (200 reactions):

- **80 µl** **TaqStart™ Antibody** (7 µM; 1.1 µg/µl) in storage buffer:
  50 mM KCl, 10 mM Tris-HCl (pH 7.0), 50% glycerol
- **1.0 ml** **Dilution buffer:**
  50 mM KCl, 10 mM Tris-HCl (pH 7.0)

Cat. No. 639251 (500 reactions):

- **200 µl** **TaqStart™ Antibody** (7 µM; 1.1 µg/µl) in storage buffer
- **2 x 1.0 ml** **Dilution buffer**

III. Additional Materials Required

The following materials are required but not supplied:

- **10X PCR reaction buffer**

Tris-HCl	100 mM
KCl	500 mM
$MgCl_2$	15 mM
pH 8.3 (at 25°C)	

Note: Variations in the concentration of $MgCl_2$, or the addition of gelatin, should not affect the performance of the TaqStart Antibody.

- **dNTP mixture** [dATP, dCTP, dGTP, & dTTP; 10 mM final concentration of each dNTP in mixture]
- **Sterile, distilled water**
- ***Taq* DNA polymerase**

IV. TaqStart™ Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

A. General Considerations

1. Storage and use

   In the supplied storage buffer (containing 50% glycerol) and at the supplied concentration (>1 µg/µl), TaqStart Antibody will not freeze at –20°C. However, repeated freezing and thawing of diluted TaqStart Antibody may adversely affect its function. There are two basic approaches to using TaqStart Antibody in order to avoid this problem:

   a. A portion of the TaqStart antibody can be diluted to a working concentration and mixed with *Taq* DNA polymerase immediately prior to use, as described in Section IV.B. The diluted portion may be stored at 4°C for ≤3 months. Store the undiluted portion of the TaqStart Antibody at –20°C. This alternative is recommended if you wish to retain the option of using different molar ratios of TaqStart Antibody to *Taq* DNA polymerase for different experiments.

   b. TaqStart Antibody can be added directly (i.e., without first diluting) to a batch of *Taq* DNA polymerase, aliquoted, and stored at –20°C for later use, as described in Section IV.C. This alternative is more convenient than the above alternative if you plan to use the same molar ratio of TaqStart Antibody to *Taq* DNA polymerase for a number of experiments.

2. Choice of enzymes

   TaqStart Antibody has been developed to bind to and inactivate DNA polymerase of the *Thermus aquaticus* YT1 strain, and will function well with commercially available *Taq* DNA polymerases licensed for use in PCR, using a molar ratio of 28:1 (antibody:polymerase). DNA polymerases of species other than *T. aquaticus* are not likely to benefit from use of TaqStart Antibody. Some genetically altered forms of *Taq* DNA polymerase may have significantly different specific activities, mutated binding sites, or other factors that may require different molar ratios for optimal results. Thus, it may be necessary to titrate the TaqStart Antibody amount (relative to the amount of polymerase) before you start your experiments (see Section V.B).

3. Use of additives

   TaqStart Antibody binds *Taq* DNA polymerase with high affinity under the conditions stated in this protocol. **Please note that the use of DMSO or formamide with TaqStart Antibody is not recommended due to interference with antibody function.** Other cosolvents, solutes (e.g., salts), and extremes of pH or other reaction conditions may reduce the affinity of TaqStart Antibody for the polymerase and thereby compromise the effectiveness of the antibody.

IV. TaqStart™ Protocol *continued*

4. [Optional] In all the PCR applications we have tested, we have found that TaqStart Antibody works reliably at a molar ratio of 28:1 antibody:polymerase. However, depending on the primers, target, or *Taq* DNA polymerase you are using, you may be able to obtain satisfactory results using a lower ratio of antibody to polymerase. If this is the case, you can try using two-fold serial dilutions of the working dilution (Step IV.B.1.a). Please note, however, that in most cases, TaqStart Antibody loses its effectiveness if the ratio of antibody to polymerase is below 10:1.

B. Dilution of the TaqStart™ Antibody for Immediate Use in PCR

1. Dilution of TaqStart Antibody and preparation of PCR master mix

   a. Prepare a working dilution of TaqStart Antibody using the supplied dilution buffer. The dilution described here will supply enough TaqStart Antibody to prepare a PCR master mix for 10 PCR amplifications (using a 50 µl reaction volume).

      4.4 µl TaqStart Antibody (1.1 µg/µl; 7 µM)
      17.6 µl Dilution buffer
      22.0 µl TaqStart Antibody (0.22 µg/µl; 1.4 µM)

   b. Mix the diluted TaqStart Antibody with *Taq* DNA polymerase. We recommend a dilution of 28 (molar) parts TaqStart Antibody to 1 (molar) part *Taq* DNA polymerase. If prepared as described here (i.e., using a molar ratio of 28:1), the mixture will be sufficient for 10 PCR reactions, each using 2.4 µl of the mixture per reaction.

      22.0 µl TaqStart Antibody working dilution (0.22 µg/µl; 1.4 µM)
      4.4 µl *Taq* DNA polymerase (5 units/µl or 0.25 µM)
      26.4 µl Total

      Note: This mixture of TaqStart Antibody and *Taq* DNA polymerase will freeze at –20°C, but it can be stored at 4°C for ≤3 months for later use. See Section IV.C if you want to prepare aliquots of premixed TaqStart and *Taq* DNA polymerase that can be stored at –20°C for later use.

   c. Incubate the mixture of TaqStart Antibody and *Taq* DNA polymerase at room temperature (20–22°C) for 5 min before assembling PCR reactions. The TaqStart Antibody/*Taq* DNA polymerase mixture can be incubated for up to 30 min with no deleterious effects.

   d. We recommend using a master mix to minimize tube-to-tube variation in PCR. Table I at the top of the next page describes PCR Master Mix I—a typical master mix for 10 PCR tubes, based on a final reaction volume of 50 µl per tube.

IV. TaqStart™ Protocol *continued*

TABLE I: PCR MASTER MIX I

Reagent	Per rxn	For 10 rxns (+ 10% extra)	Final Conc.*
10X PCR reaction buffer	5 µl	55 µl	
20 µM 5' primer	1 µl	11 µl	0.40 µM
20 µM 3' primer	1 µl	11 µl	0.40 µM
dNTP mixture [10 mM each dNTP]	1 µl	11 µl	0.20 mM (each)
dH_2O	37.6 µl	413.6 µl	
Freshly prepared 28:1 mixture of TaqStart Antibody + *Taq* DNA polymerase	2.4 µl	26.4 µl	0.056 µM 0.002 µM
Total volume	48 µl	528 µl	

* Final concentration of components in the reaction mixture, based on a 50 µl final reaction volume.

e. Combine your cDNA or DNA sample with PCR Master Mix I as follows:

48 µl PCR Master Mix I
2 µl cDNA or DNA sample
50 µl total

f. If necessary, add approximately 50 µl of mineral oil to each tube to prevent evaporation during thermal cycling.

2. PCR thermal cycling

Use the DNA thermal cycling program you normally use. Please note, however, that greater yields and/or sensitivity may result from the use of TaqStart Antibody; therefore, fewer temperature cycles may be needed to achieve the same yield. In the case of PCR reactions using extremely low-copy-number target sequences, additional cycles (up to a total of 40–45) may be used to generate enough product to visualize by ethidium bromide staining after gel electrophoresis.

IV. TaqStart™ Protocol *continued*

C. Preparing, Storing, and Using Aliquots of Premixed TaqStart™ Antibody and *Taq* DNA Polymerase

The concentrated TaqStart™ Antibody may be added directly to an aliquot of *Taq* DNA polymerase. The mixture of TaqStart Antibody and *Taq* DNA polymerase may then be aliquoted and stored at –20°C for up to 6 months. This is convenient if you plan to use the same molar ratio of TaqStart Antibody to *Taq* DNA polymerase for a number of experiments.

Note: If the mixture is prepared as described in Step 1.a below, the mixture will not freeze when stored at –20°C, due to the high concentration of glycerol (50%, if the polymerase is also in a storage buffer containing 50% glycerol).

1. Mixing, aliquoting, and storing TaqStart Antibody/*Taq* DNA polymerase
   a. Add one volume of TaqStart Antibody to one volume of *Taq* DNA polymerase. The example below gives the reagent amounts sufficient for 1 batch of PCR Master Mix II (10 PCR amplifications + 10% extra), which is described in Step 2 below. These volumes can be scaled up if you are planning to aliquot and store the premixed TaqStart Antibody/*Taq* DNA polymerase.

      4.4 µl TaqStart Antibody (1.1 µg/µl; 7 µM)

      4.4 µl *Taq* DNA polymerase (5 units/µl; 0.25 µM)

      8.8 µl Total mixture

   b. Incubate the mixture of TaqStart Antibody and *Taq* DNA polymerase at room temperature (20–22°C) for 5 min before aliquoting and storing or adding it to PCR Master Mix II (Step 2.a below). The TaqStart Antibody/*Taq* DNA polymerase mixture can be incubated for up to 30 min with no deleterious effects.
   c. If desired, aliquot the mixture of TaqStart Antibody and *Taq* DNA polymerase for use at a later date. Aliquots prepared this way can be stored at –20°C for up to 3 months.
2. Use of premixed TaqStart Antibody/*Taq* DNA polymerase in PCR
   a. We recommend using a master mix to minimize tube-to-tube variation in PCR. The prealiquoted TaqStart Antibody/*Taq* DNA polymerase mixture can be added directly to a PCR master mix. Table II at the top of the next page describes PCR Master Mix II—a typical master mix for 10 PCR tubes, based on a final reaction volume of 50 µl per tube. Unlike PCR Master Mix I (Table I), PCR Master Mix II uses the premixed TaqStart Antibody/*Taq* Polymerase prepared above in Step IV.C.1.

IV. TaqStart™ Protocol *continued*

TABLE II: PCR MASTER MIX II

Reagent	Per rxn	For 10 rxns (+ 10% extra)	Final Conc.*
10X PCR reaction buffer	5 µl	55 µl	
20 µM 5' primer	1 µl	11 µl	0.40 µM
20 µM 3' primer	1 µl	11 µl	0.40 µM
dNTP mixture [10 mM each dNTP]	1 µl	11 µl	0.20 mM (each)
dHO	39.2 µl	431.2 µl	
Concentrated, premixed TaqStart Antibody + *Taq* DNA polymerase	0.8 µl	8.8 µl	0.056 µM 0.002 µM
Total volume	48 µl	528 µl	

* Final concentration of components in the reaction mixture, based on a 50 µl final reaction volume.

b. To assemble PCR reactions, combine your cDNA or DNA sample with PCR Master Mix II as follows:

48 µl PCR Master Mix II
2 µl cDNA or DNA sample
50 µl Total

c. If necessary, add approximately 50 µl of mineral oil to each tube to prevent evaporation during thermal cycling.

d. Commence thermal cycling as described in Step IV.B.2.

V. Troubleshooting Guide

The simplicity of the TaqStart Antibody system makes its use fairly straightforward. If a particular system is helped by the hot start PCR method, it is likely to be helped by the TaqStart Antibody. Consequently, if no reduction of nonspecific products is observed when using TaqStart Antibody, the first thing to do is to test the PCR system with a conventional hot start method.

A. Both TaqStart™ PCR and the Conventional Hot Start PCR Yield Multiple Nonspecific Products.

1. Raise the annealing temperature in 2–3°C increments. Raising the temperature will improve the binding specificity of the primers, but it may also result in reduced primer binding and extension. If raising the annealing temperature causes a reduced yield of the specific product with only a proportional reduction of side reaction products, it may be necessary to redesign the primers (Huang & Jeang, 1994).
2. Take special precautions to avoid crossover contamination of PCR reactions with both specific and nonspecific PCR products, including primer-dimer artifacts (Kwok & Higuchi, 1989).

B. TaqStart™ PCR Yields More Nonspecific Products Than Conventional Hot Start PCR.

Titration of the TaqStart Antibody may be necessary to achieve the same degree of improvement as with a conventional hot start, especially if you are using a modified *Taq* DNA polymerase (see Section IV.A.2), or if you are using PCR reaction conditions other than those suggested in this protocol. If you feel such an antibody titration is necessary, we suggest starting with a working dilution (Step IV.B.1) that has a twofold to fourfold higher concentration of TaqStart Antibody than that recommended in the protocol.

C. Yield of Specific Product Is Low Using TaqStart™ Antibody.

1. If a greater yield is necessary, you may scale up the reaction volume to 100 or 150 µl or more so that more of the product is generated.
2. You may increase the number of amplification cycles. If you are using 25–30 cycles and obtaining low yields, increasing the cycle number to 35–40 should result in higher yields without significantly increasing side reaction products.
3. Modify reaction conditions and/or selection of PCR targets to obtain greater opportunities for primer annealing. For example, increase the denaturation time up to 1–1.5 min and/or increase the denaturation temperature to as high as 95°C to overcome denaturation difficulties. Extremely GC-rich regions should be avoided as PCR targets, if possible. **Please note that the use of DMSO or formamide with TaqStart Antibody is not recommended, due to interference with antibody function.** Alternatively, reduce the annealing temperature in

V. Troubleshooting Guide *continued*

2–3°C increments or increase the $MgCl_2$ concentration in 0.5 mM increments to increase yield without additional side reaction products. This approach may be especially helpful with primers that have bases mismatched to the target sequence.

4. Increasing the pH of the reaction in 0.3 unit increments (to a maximum of pH 9.2) or increasing the concentration of KCl to 75 mM may increase the yield of specific PCR products in some cases.
5. If TaqStart Antibody is used at a concentration greater than fivefold more than that recommended in the protocol, there is a risk that the excess antibody or glycerol from the storage buffer may inhibit the reaction. If this is the case, antibody titration may help alleviate the problem.

VI. References

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VII. Related Products

For a complete listing of all Clontech products, please go to
www.clontech.com

Products	Cat. No.
• TITANIUM™ *Taq* DNA Polymerase	639208 639209
• PowerScript™ Reverse Transcriptase	639500 639501
• Advantage™ RT-for-PCR Kit	639505 639506
• TITANIUM™ One-Step RT-PCR Kit	639503 639504
• RT-PCR Amplimer Sets	many

Notes

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