Clontech Laboratories, Inc.

Titanium® *Taq* PCR Kits User Manual

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I. Introduction

Titanium *Taq* DNA Polymerase is a highly sensitive, robust enzyme for use in all PCR applications. It is ideal for the PCR amplification of any DNA template, including bacterial and plasmid DNA, cDNA, and complex genomic DNA. Titanium *Taq* contains **TaqStart® Antibody**, which provides an integrated hot start for increased specificity and yield (Kellogg *et al.*, 1994).

Titanium *Taq* DNA Polymerase lacks the 5'- exonuclease activity of wild-type *Taq* (Kim *et al.*, 1995), which makes it more robust than other *Taq* polymerases and allows it to amplify highly complex DNA mixes. This novel enzyme also contains carefully engineered amino acid substitutions that increase its solubility, making it the most sensitive PCR polymerase available (Barnes, 1992).

A. Major Advantages over other DNA Polymerases

- Increased sensitivity allows you to amplify your target using fewer PCR cycles while reducing background—and amplify rare or low copy targets when other polymerases fail.
- Perform PCR without optimizing your reaction conditions—Titanium *Taq* tolerates a wide range of magnesium concentrations, and is supplied with a pre-optimized buffer.
- Obtain exceptionally high yields when amplifying targets of up to 2 kb from highly complex templates, such as mammalian genomic DNA. Targets of up to 4 kb can be readily amplified when the template is a moderately abundant cDNA or a less complex genomic DNA.
- An integrated hot start antibody allows room temperature reaction set-up and increased specificity.

B. Integrated Hot Start for Higher Specificity

Titanium *Taq* comes preblended with TaqStart Antibody. This hot start antibody, which is quickly inactivated at the onset of thermal cycling, blocks polymerase activity during the PCR reaction set-up, preventing the formation of primer-dimers and other artifacts prior to thermal cycling. This integrated hot start feature allows for higher specificity than other traditional *Taq*-based methods. It is especially useful for multiplex PCR because it limits the number of mispriming events caused by multiple primer sets, which can result in spurious PCR products or smeared gel bands.

C. Applications of Titanium® *Taq*

Titanium *Taq* is designed for most routine PCR applications. The kits can be used for analytical PCR, RT-PCR, insert screening, multiplex PCR, and primer extension procedures.

If you plan to amplify long (>5 kb) and/or highly complex templates for preparative purposes or for specialized applications, we recommend our **Advantage® 2 Mixes and Kits**. If high fidelity is of primary importance, use **Advantage HF 2**. If amplifying GC-rich sequences, use **Advantage GC 2**.

II. List of Components

Titanium® *Taq* PCR Kit (Cat. Nos. 639210 & 639211)

Store all components at –20°C. Enough reagents are supplied for 30 or 100 PCR reactions of 50 µl each.

<u>30 rxns</u>	<u>100 rxns</u>	
30 µl	**100 µl**	**50X Titanium® *Taq* DNA Polymerase (includes TaqStart Antibody)**

Concentration in 50X Mix	Component	Final Rxn Concentration
50%	Glycerol	1.0%
20 mM	Tris-HCl (pH 8.0)	0.4 mM
100 mM	KCl	2 mM
0.1 mM	EDTA (pH 8.0)	2.0 µM
0.25%	Tween-20	0.005%
0.25%	Nonidet P-40	0.005%

200 µl	**600 µl**	**10X Titanium® *Taq* Buffer**

Concentration in 10X Mix	Component	Final Rxn Concentration
400 mM	Tricine-KOH (pH 8.0 at 25°C)	40 mM
160 mM	KCl	16 mM
35 mM	$MgCl_2$	3.5 mM
37.5 µg/ml	BSA	3.75 µg/ml

40 µl	**120 µl**	**50X dNTP Mix** (10 mM each of dATP, dCTP, dGTP, and dTTP; final rxn concentration: 0.2 mM each
30 µl	**100 µl**	**Control DNA Template** (100 ng/µl) Calf Thymus DNA
30 µl	**100 µl**	**Control Primer Mix** (10 µM each) The sequences are: 5' primer 5'–CTTGTCCTAATCTTCCTCCTCACGGCA–3' 3' primer 5'–TGGCACGGCCATAAGAGGTAGATGTCA–3'
2.5 ml	**5.0 ml**	**PCR-Grade Water**

III. Additional Materials Required

The following reagents are not supplied.

- [Optional] **Mineral oil** (We recommend Sigma Cat. No. M-3516.)
- **PCR reaction tubes**
- **Thermal cycler**
- **Dedicated pipettors**
- **PCR pipette tips** suitable for the above pipettors and preferably equipped with hydrophobic filters.
- **DNA size markers** (See Section V.C)
- **5X Stop/loading buffer** (Sambrook & Russell, [2001] provides several recipes.)

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. Titanium *Taq* can be used in a wide variety of PCR applications. Although the constraints on primer design will vary from one application to the next, the following general guidelines are recommended:

- **Length:** Primers should have a T_m of ~70°C to achieve optimal results in a two-step cycling program with a 68°C combined annealing/extension step. Therefore, whenever possible, primers should be at least 22 nucleotides long (25–30-mers are preferred)
- **GC Content:** Primers should have a GC content of 45–60%. Furthermore, the 3' ends of each primer should not be complementary to each other and should have a low GC content.

Important: Always check and recheck your primer design before constructing or ordering primers.

B. Template Quality

The following factors should be considered when preparing template for PCR reactions:

- Many conventional PCR applications using Titanium *Taq* work well with templates of average or even low quality, because PCR amplification proceeds exponentially.
- The longer or more complex the target, or the more sensitive the application, the more important template quality becomes.
- Large targets require high-quality, high molecular weight DNA because some depurination occurs when DNA is denatured during thermal cycling, which can lead to truncated products.

C. Good PCR Practices

1. **Use dedicated pipettors in a dedicated work space** to avoid nonspecific amplification.
   a. Use small aliquots of starting material to avoid contaminating your stocks.
   b. Wear gloves and use PCR pipette tips with hydrophobic filters and dedicated solutions.
2. **Careful pipetting of small volumes** is necessary to avoid tube-to-tube variation.
   a. Always be sure that there is no extra solution on the outside of the pipette tip before transfer.
   b. When adding solution to a tube, immerse the tip into the reaction mixture and deliver the contents from the pipette tip into the mixture, then rinse the tip by pipetting up and down several times.
3. **Use a Master Mix**, which contains the appropriate volumes of all reagents required for multiple PCR reactions, to save time and greatly reduce tube-to-tube variation.
   a. If multiple templates are being tested with the same primers, include primers in the Master Mix.
   b. If one template is being tested with multiple primer sets, include the template in the Master Mix.
   c. If you are setting up several sets of parallel samples, assemble multiple Master Mixes (e.g., each with a different set of primers).
   d. The Master Mix should be **gently but thoroughly mixed before use (i.e., pulse vortex to prevent bubble formation).**
4. **PCR Control Reactions:** include positive and negative controls (e.g., H_2O instead of DNA template).

D. Additional Specifications & Applications

1. **Touchdown PCR:** We have found that "touchdown" PCR significantly improves the specificity of many PCR reactions in a wide variety of applications (Don *et al.*, 1991; Roux, 1995). See Section VII.B for more information.
2. **Half-Life:** The half-life of Titanium *Taq* depends on the specific reaction conditions used, but generally ranges from 20–40 min at 95°C.
3. **Effect of Additives on TaqStart Antibody and *Taq* DNA Polymerase Function**
   a. TaqStart Antibody binds Titanium *Taq* Polymerase with high affinity under the conditions described in this protocol.
   b. The addition of 2–5% DMSO will not interfere with TaqStart function and may improve results in some instances. However, adding formamide or other cosolvents may disrupt TaqStart function.
   c. Excessive glycerol, solutes (e.g., salts), pH extremes, or other deviations from the recommended reaction conditions may reduce the effectiveness of TaqStart Antibody and/or *Taq* DNA Polymerase.
4. **Titanium *Taq* is NOT Recommended for the Following Applications**
   a. Mutagenesis protocols involving so-called "sloppy" PCR
   b. Highly sensitive applications such as SMART™ PCR cDNA Synthesis, SMART cDNA Library Construction, or PCR-Select™ cDNA Subtraction

5. **T/A Cloning:** Titanium *Taq* PCR products are compatible with T/A cloning methods. For best results, please observe the T/A cloning tips in Section V.B.

V. Protocols

A. PCR Control Reactions Protocol

The following PCR control reactions can be performed in parallel with your experiments to ensure that Titanium *Taq* is working properly. A positive-control template and primers are provided in the Titanium *Taq* PCR Kit. When using Titanium *Taq* with other Clontech kits, use the positive controls provided with those kits.

1. Allow all components to thaw completely on ice and mix each thoroughly before use.
2. Combine the following reagents in a PCR tube.

	Reagent Volume (µl per sample)	
Reagent	**Positive Control**	**Negative Control**
PCR-Grade H_2O	41	42
10X Titanium *Taq* PCR Buffer	5	5
Control DNA template (100 ng/µl)	1	—
Control Primer Mix (10 µM each)	1	1
50X dNTP mix (10 mM each of dATP, dCTP, dGTP, dTTP)	1	1
50X Titanium *Taq* DNA Polymerase	1	1
Total volume per rxn	50	50

3. Mix well and spin the tube briefly to collect all the liquid in the bottom of the tube.
4. If your thermal cycler does not have a "hot lid", add 1–2 drops of mineral oil to each PCR reaction to prevent evaporation during cycling. A good "seal" of mineral oil should have a well-defined meniscus between the two phases. Cap the PCR tubes firmly.
5. Begin thermal cycling using the following parameters (optimized for the control template and primers included in the kit) or the general cycling parameters described in Section V.B:
    - 95°C for 1 min
    - 30 cycles
        95°C for 30 sec
        68°C for 1.5 min
    - 68°C for 1.5 min
6. Transfer 5 µl of your PCR reaction to a fresh tube and add 1 µl of 5X stop/loading buffer. Analyze your samples, along with suitable DNA size markers, on a 1.2% agarose/EtBr gel. See Section V.C for more information.

Expected results: The positive control reagents provided in the kit should amplify a 407 bp portion of the bovine pancreatic trypsin inhibitor gene, which will be seen as a single band on an agarose gel. No products should be produced by the negative (e.g., no template) control.

B. Recommended Cycling Parameters

Use the following general guidelines when setting up your initial experiments with Titanium *Taq*. The optimal parameters may vary with different thermal cyclers and will depend on your particular primers, templates, and other experimental variables.

NOTE: If you intend to capture your PCR product by T/A cloning, we recommend that you add an additional 10 min extension at 70°C, and then immediately clone or freeze the PCR product. Do not store the reaction at 4°C. These steps will help ensure the incorporation and preservation of 3' A-overhangs

Target Size	Cycle Parameters
<5 kb:	• 95°C for 1 min • 25–35 cycles[A] 95°C for 30 sec[B] 68°C for 1–3 min[C] • 68°C for 3 min[D]
>5 kb	Use Advantage 2 Kit

[A] Use 25 cycles for multiple-copy genes or medium-to-high abundance cDNAs and 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)—unless the T_m of the primers is <60–65°C and in certain special protocols.

[B] Use the shortest possible denaturation time. In some cases, better results may be obtained by modifying the denaturation step (15 sec, 94°C). 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. Minimizing denaturation time is particularly important in experiments with very large templates where total cycling time can exceed 12 hr.

[C] Use the longest possible annealing/extension temperature. See Note A. Some researchers prefer to use an annealing/extension time equal to the expected target size (in kb) plus two minutes. We recommend using 1 min per kb of expected target.

[D] Optional: This final extension may reduce background in some cases.

C. Analyzing PCR Reactions Using Agarose Gel Electrophoresis

Use the following conditions to analyze a 5 µl sample of your PCR reaction on an agarose gel. The remaining 45 µl of the reaction mixture can be subjected to further cycling if you do not see a product.

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. References

Barnes, W. M. (1992) The fidelity of *Taq* polymerase catalyzing PCR is improved by an N-terminal deletion. *Gene* **112**(1):29–35.

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**(14):4008.

Kellogg, D. E., Rybalkin, I., Chen, S., Mukhamedova, N., Vlasik, T., Siebert, P. & Chenchik, A. (1994) TaqStart Antibody: Hotstart PCR facilitated by a neutralizing monoclonal antibody directed against *Taq* DNA polymerase. *BioTechniques* **16**(6):1134–1137.

Kim, Y., Eom, S. H., Wang, J., Lee, D. S., Suh, S. W. & Steitz, T. A. (1995) Crystal structure of *Thermus aquaticus* DNA polymerase. *Nature* **376**(6541):612–616.

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**(1):125–128.

Roux, K. H. (1995) Optimization and troubleshooting in PCR. *PCR Methods Appl.* **4**(5):S185–S194.

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

VII. Troubleshooting Guide

The following **general** guidelines apply to most PCR reactions. However, no attempt has been made to address troubleshooting for all of the many applications for which Titanium *Taq* can be used. When using the enzyme with one of its Clontech companion products, additional, application-specific troubleshooting information can be found in the relevant User Manual.

A. No Product Observed

Description of Problem	Solution
PCR component is missing or 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.
Too few cycles	Increase the number of cycles (3–5 additional cycles at a time).
Annealing temperature is too high	Decrease the annealing temperature in increments of 2–4°C.
Suboptimal primer design	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 GC content of less than 45%, try to design a primer with a GC content of 45–60%.
Not enough template	Repeat PCR using a higher concentration of DNA (after trying more cycles).
Poor template quality	Check template integrity by electrophoresis on a standard TBE-agarose gel. If necessary, repurify your template using methods that minimize shearing and nicking.
Denaturation temperature is too high or too low	Optimize denaturation temperature by decreasing or increasing it in 1°C increments. (A denaturation temperature that is too high can lead to degradation of the template, especially for long target sequences.)

A. No Product Observed (*continued*)

Description of Problem	Solution
Denaturation time is too long or too short	Optimize denaturation time by decreasing or increasing it in 10 sec increments. (A denaturation time that is too long can lead to degradation of the template, especially for long target sequences.)
Extension time is too short	(Especially with longer templates) Increase the extension time in 1 min increments
Too little enzyme	Titanium *Taq* is supplied at 50X for most applications. Therefore, try to optimize the cycle parameters as described above before increasing the enzyme concentration. In rare cases, the yields can be improved by increasing the concentration of the enzyme mix. However, increasing the concentration >2X is likely to lead to higher background levels.
[Mg^{2+}] is too low	Titanium *Taq* performs well at a broad range of Mg^{2+} concentrations. Therefore, as long as you use the buffer included with the 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 to below a minimum level.
[dNTPs] is too low	When used as recommended, the 50X dNTP Mix provided with the kit gives a final concentration of 0.2 mM of each dNTP. In our experience, this concentration of dNTPs is suitable for a wide range of applications. • If you are preparing your own dNTPs, be sure that the final concentration of each dNTP in the reaction is 0.2 mM. • Some manufacturers recommend using concentrations higher than 0.2 mM of each dNTP when amplifying large templates. However, we have had no trouble amplifying large templates using 0.2 mM of each dNTP. We have gone up to 35 kb with the Advantage Genomic PCR Kit, so it is unlikely that dNTPs are limiting. • Note that if you increase the concentration of dNTPs, you will also need to increase the Mg^{2+} concentration proportionately.
Dificult target	If you are amplifying a complex template, a genomic template, a template greater than 5 kb, or a template with a high GC content, and get poor results using Titanium *Taq*, use one of our Advantage products (see Section I.C).

B. Multiple Products

Description of Problem	Solution
Too many cycles	Reducing the cycle number by 3–5 cycles may eliminate nonspecific bands.
Annealing temperature is too low	Increase the annealing/extension temperature in increments of 2–3°C.
Suboptimal primer design	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 GC content of less than 45%, try to design a primer with a GC content of 45–60%.
Touchdown PCR needed	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications (Don *et al.*, 1991; Roux, 1995). Touchdown PCR involves using 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 VII.D.

C. Products are Smeared on Gel

Description of Problem	Solution
Too many cycles	Reducing the cycle number by 3–5 cycles may eliminate nonspecific bands.
Denaturation temperature is too low	Try increasing the denaturation temperature in increments of 1°C.
Extension time is too long	Decrease the extension time in 1–2-min increments.
Poor template quality	Check template integrity by electrophoresis on a denaturing agarose gel. Repurify your template if necessary.
Touchdown PCR is needed	See "Touchdown PCR needed" in Section VII.B.
Too much enzyme	Titanium *Taq* is supplied at 50X for most applications; however, a 1X final concentration of the enzyme mix may be too high for some applications. If smearing is observed, first try optimizing the cycle parameters as described above, then try reducing the enzyme concentration to 0.5–0.2X.
Too much template	Try a lower concentration of DNA template in the PCR reaction.
Contamination	See Section VII.D.

D. Dealing with Contamination

Contamination most often results in extra bands or smearing. It is important to include a negative control (containing primers but no template) 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 the PCR reaction and perform the post-PCR analysis in separate laboratory areas with separate sets of pipettors.
- 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 advise using commercially available aerosol-free pipette tips.
- 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.

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