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ProductInformation

TaqStart™ Antibody

Product Code **T 4809**
Technical Bulletin No. MB-375
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

TaqStart™ Antibody provides a simple alternative to several methods that are collectively called "hot start" PCR†.[1-3] These methods all aim at preventing Taq Polymerase activity in the reaction mix prior to the first high temperature step in the PCR cycle. TaqStart Antibody is a neutralizing monoclonal antibody to Taq DNA polymerase.[4-6] When the antibody is bound to Taq, the enzyme is rendered inactive. The antibody is bound to the enzyme prior to the assembly of the reaction mix. The binding of the antibody to the enzyme takes only 10 minutes. The inhibition of Taq DNA polymerase is completely reversed when the temperature is raised above 90 °C. At the first denaturation step in thermal cycling, the enzyme-antibody complex dissociates and the TaqStart Antibody becomes nonfunctional. Once the antibody is denatured, the activity of the Taq DNA polymerase is restored and the enzyme functions normally during the course of the PCR reaction. TaqStart Antibody is effective with a variety of commercially available Taq DNA polymerases.

Hot start PCR has been proven to reduce generation of nonspecific amplification products and primer-dimer artifacts. Typical applications for TaqStart Antibody include PCR reactions involving one or more of the following: complex genomic or cDNA templates, very low-copy-number targets, large number of thermal cycles (>35) and multiple primer pairs in the same reaction tube.

Reagents Provided

- TaqStart Antibody, Product Code T 8559
  1.1 µg/µl (7 µM) in 50 mM KCl, 10 mM Tris-HCl, pH 7.0, 50% glycerol. Provided as 200 reactions (80 µl) or 500 reactions (200 µl).

- Dilution Buffer for TaqStart Antibody, Product Code D 4425
  50 mM KCl, 10 mM Tris-HCl (pH 7.0) provided in 1 ml vials.

Reagents and Equipment Required but Not Provided
(Sigma Product Numbers have been given where appropriate.)

- Taq DNA polymerase, Product Code D 1806
- 10X PCR Buffer, Product Code P 2192
- Deoxynucleotide Mix, Product Code D 7295
  10 mM dATP, 10 mM dCTP
  10 mM dGTP, 10 mM TTP*
- Water, PCR Reagent, Product Code W 1754
- Mineral Oil, Product Code M 8662 (optional)
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Product Codes P 3114 and P 3364
- Thermocycler
- Primers
- DNA to be amplified
- Chloroform, Product Code C 7559 (optional)

* Note: The Deoxynucleotide Mix may be replaced with these individual nucleotides, 10 mM dATP, Product Code D 6920, 10 mM dCTP, Product Code D 7045, 10 mM dGTP, Product Code D 7170, and 10 mM TTP, Product Code T 7791.

Precautions and Disclaimer

TaqStart Antibody is for R&D use only. Not for drug, household or other uses. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. Refer to Material Safety Data Sheet.

Preparation Instructions

TaqStart Antibody has been developed to bind to and inactivate DNA polymerase of *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. Some genetically altered forms of Taq DNA polymerase may have significantly different specific activities, mutated binding sites, or other factors which may require different molar

ratios for optimal results. In these instances, it may be necessary to titrate the TaqStart Antibody relative to the polymerase before starting experimentation.

Note: The use of DMSO or formamide with TaqStart Antibody is not recommended due to interference with the hot start function. Other cosolvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of TaqStart Antibody for the polymerase and thereby compromise the effectiveness of the antibody.

Dilution of the TaqStart Antibody for immediate use in PCR

1. Prepare a working solution of the TaqStart Antibody using the supplied dilution buffer. The dilution described below will provide enough antibody:enzyme complex for 10 PCR amplifications of 50 or 100 µl each plus 10% extra.

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

2. Mix the diluted TaqStart Antibody with Taq DNA polymerase. A dilution of 28 (molar) parts of TaqStart Antibody to 1 (molar) part Taq DNA polymerase is suggested.

   22.0 µl Diluted TaqStart Antibody (0.22 µg/µl; 1.4 µM)
   4.4 µl Taq DNA polymerase (5 units/µl; 0.25 µM)
   26.4 µl total

3. Incubate the mixture for 10 minutes at room temperature. The mixture can be incubated for up to 30 minutes at room temperature with no effect on performance. The Taq DNA Polymerase:TaqStart Antibody conjugate may be stored at 4 °C for up to 3 months.

4. If prepared as described above, the mixture will be enough for 10 PCR reactions using 2.4 µl of the antibody:enzyme conjugate per reaction.

Storage/Stability
Store at –20 °C.
In the supplied storage buffer and at the supplied concentration, TaqStart Antibody will not freeze at –20 °C. Repeated freeze-thaw of diluted TaqStart Antibody may adversely affect its function. TaqStart Antibody diluted to a working concentration in the dilution buffer provided may be stored at 4 °C for up to 3 months. The diluted antibody should only be mixed with Taq DNA polymerase immediately prior to use.

Procedure
A. Preparation of PCR Master Mix and Thermal Cycling Parameters

Because Taq DNA polymerase is a magnesium ion-dependent enzyme, the optimal conditions for the concentration of Taq DNA polymerase, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the Taq DNA polymerase, cycling parameters, and the $MgCl_2$ concentration. The use of DMSO or formamide with TaqStart antibody is not recommended due to interference with the TaqStart function.

To minimize tube-to-tube variation, preparation of a PCR master mix is recommended. The amount prepared should be based on the number of PCR reactions to be performed.

1. For a single reaction, add the following reagents to a 0.2 or 0.5 ml PCR tube in the following order:

q.s.	Water (for a final volume of 100 µl)
100 µl	10X PCR Buffer
2 µl*	Deoxynucleotide Mix
2 µl	Primer 1, 0.1-1.0 µM (typically 15-30 bases in length)
2 µl	Primer 2, 0.1-1.0 µM (typically 15-30 bases in length)
2.4 µl	Taq DNA Polymerase:TaqStart Antibody conjugate
x µl	Template DNA (typically 10 ng)
100 µl	Total volume

*Note: The individual nucleotides (2 µl of each 10 mM solution (8 µl total) may be substituted for the Deoxynucleotide Mix, Product Code D 7295,

2. Mix gently and briefly centrifuge to collect all components to the bottom of the tube.

3. Add 100 µl of mineral oil to the top of each tube to prevent evaporation.

4. The amplification parameters will vary depending on the primers and the thermocycler used. It may be necessary to optimize the system for individual primers, template, and thermocycler.

Common cycling parameters:

a. Denature the template at 94 °C for 1 minute
b. Anneal primers at 55 °C for 2 minutes
c. c. Extension at 72 °C for 3 minutes

25-30 cycles of amplification are recommended.

5. Agarose gel electrophoresis and subsequent ethidium bromide staining can evaluate the amplified DNA. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

B. 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. This mixture may then be aliquoted and stored at –20 °C for up to 6 months.

1. Add one volume of TaqStart Antibody to one volume of Taq DNA polymerase. Described below is the reagent amounts for 10 PCR amplifications plus 10% extra.

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 volume

2. Incubate the mixture for 10 minutes at room temperature. The mixture can be incubated for up to 30 minutes with no effects on performance.

Note: This mixture can be scaled up, aliquoted to multiple tubes and stored at –20 °C for up to 6 months.

3. If prepared as described above, the mixture is enough for 10 PCR reactions using 0.8 µl of the antibody:enzyme conjugate per reaction. The same reaction mix is prepared as above with the difference in volume for the antibody:enzyme conjugate being made up with water. Please note the difference in volume from the TaqStart Antibody diluted for immediate use.

Troubleshooting Guide

1. If no reduction of nonspecific products is observed when using TaqStart Antibody, test the PCR system using a conventional hot start method. If both the TaqStart PCR and the conventional hot start PCR yield multiple nonspecific products:
   a. Raise the annealing temperature in 2-3 °C increments. Raising the temperature improves the specificity of binding by the primers, however, it may result in reduced binding and extension of the primers. 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.[7]
   b. Take special precautions to avoid crossover contamination of PCR reactions with both specific and nonspecific PCR products, including primer-dimer artifacts.[8]

2. If the 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. This is especially true if a modified Taq DNA polymerase is being used or the PCR reaction conditions vary from those described in this document. In this case, start with a working solution that has a two- to four-fold higher concentration of TaqStart Antibody than recommended.

3. If the yield of specific product is low using TaqStart Antibody:

   a. Increase the reaction volume to 150 µl or more.

   b. Increase the number of amplification cycles. If currently using 25-30 cycles, increase the cycle number to 35-40. This should increase yields without significantly increasing side reaction products.

   c. Modify the reaction conditions and/or selection of PCR targets to obtain greater opportunities for PCR priming. For example, increase the denaturation time up to 1-1.5 minutes and/or increase the denaturation temperature to as high as 95 °C to overcome denaturation difficulties.

Note: The use of DMSO or formamide with TaqStart antibody is not recommended due to interference with the TaqStart function.

4. If the TaqStart Antibody is used at a concentration greater than 5-fold more than recommended in the protocol, the excess antibody or glycerol from the storage buffer may inhibit the reaction. Titration of the TaqStart Antibody may be necessary to alleviate the problem.

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.
TaqStart is a trademark of CLONTECH Laboratories, Inc.

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