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Product Information

JumpStart™ *Taq* Antibody

Catalog Number **A7721**
Storage Temperature –20 °C

Product Description

JumpStart *Taq* Antibody provides an efficient method for "hot start" PCR†. JumpStart *Taq* Antibody is a neutralizing monoclonal antibody specific to *Taq* DNA polymerase[1-3]. When the antibody is bound to *Taq*, the enzyme is rendered inactive. The antibody must be bound to the enzyme prior to the assembly of the reaction mix, a process that 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 JumpStart *Taq* 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. JumpStart *Taq* Antibody is effective with a variety of commercially available *Taq* DNA polymerases. PCR using JumpStart *Taq* DNA polymerase has been proven to reduce nonspecific amplification products and primer-dimer artifacts. Typical applications for JumpStart *Taq* 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

- JumpStart *Taq* Antibody, Product Code A 5600
  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 JumpStart *Taq* Antibody, Product Code B 6808
  50 mM KCl, 10 mM Tris-HCl, pH 7.0, provided in 1 ml vials.

Reagents Required But Not Provided

(Sigma Product Codes 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, containing 10 mM dATP, 10 mM dCTP, 10 mM dGTP, and 10 mM dTTP.

or

10 mM dATP, Product Code D 6920
10 mM dCTP, Product Code D 7045
10 mM dGTP, Product Code D 7170
10 mM dTTP, Product Code T 7791
Water, Product Code W 1754
Mineral Oil, Product Code M 8662 (optional)
Thermocycler
Primers
DNA to be amplified
Chloroform, Product Code C 7559 (optional)

Precautions and Disclaimer

Sigma's JumpStart *Taq* Antibody is for R&D use only. Not for drug, household or other uses.

Preparation Instructions

JumpStart *Taq* 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 JumpStart *Taq* 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. As a result, it may be necessary to titrate the JumpStart *Taq* Antibody relative to the polymerase before starting experimentation.

Note: The use of up to 5% v/v DMSO will not interfere with the JumpStart *Taq* function. Other cosolvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of JumpStart *Taq* Antibody for the polymerase and thereby compromise the effectiveness of the antibody.

Dilution of the JumpStart *Taq* Antibody for immediate use in PCR

1. Prepare a working solution of the JumpStart *Taq* Antibody using the supplied dilution buffer. The dilution described below will provide enough antibody:enzyme complex for 12 PCR amplifications of 50 µl each plus 4% extra.

5 µl	JumpStart *Taq* Antibody (1.1 µg/µl; 7 µM)
20 µl	Dilution buffer
25 µl	Diluted JumpStart *Taq* Antibody (0.22 µg/µl; 1.4 µM)

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

25 µl	Diluted JumpStart *Taq* Antibody (0.22 µg/µl; 1.4 µM)
5 µl	*Taq* DNA polymerase (5 units/µl; 0.25 µM)
30 µl	Total volume

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:JumpStart *Taq* 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 12 PCR reactions using 2.4 µl of the antibody:enzyme conjugate per reaction.

Storage/Stability

Store at –20 °C.

As supplied, JumpStart *Taq* Antibody will not freeze at –20 °C. JumpStart *Taq* antibody diluted to a working concentration in the dilution buffer provided may be stored at 4 °C for up to 3 months. Repeated freeze-thaw of diluted JumpStart *taq* antibody may adversely affect its function. Store in frozen aliquots at –20 °C.

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 cycling parameters and $MgCl_2$ concentration.

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 200 or 500 µl microcentrifuge tube in the following order:

w µl	Water (for a final volume of 50 µl)
5 µl	10X PCR Buffer
1 µl	Deoxynucleotide Mix, Product Code D 7295*
x µl	Primer 1, 0.1-1.0 µM (typically 15 – 30 bases in length)
y µl	Primer 2, 0.1-1.0 µM (typically 15 – 30 bases in length)
2.4 µl	*Taq* DNA Polymerase:JumpStart *Taq* Antibody conjugate
z µl	Template DNA (typically 10 ng)
50 µl	Total volume

*Note: The individual nucleotides (1 µl of each 10 mM solution, 4 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 50 µl of mineral oil to the top of each tube to prevent evaporation if not using a thermocycler with a heated lid.

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.
   Typical cycling parameters:

   a. Denature the template at 94 °C for 0.5 - 1 minute.
   b. Anneal primers at 55 °C for 0.5 - 1 minute.
   c. Extension at 72 °C for 1 minute (or 1 min/kb amplicon).

   25-30 cycles of amplification are recommended.

5. Use agarose gel electrophoresis and subsequent ethidium bromide staining to 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 JumpStart *Taq* Antibody and *Taq* DNA Polymerase

The concentrated JumpStart *Taq* 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 JumpStart *Taq* Antibody to one volume of *Taq* DNA polymerase. The reagent amounts below are for 12 PCR amplifications plus 4% extra.

5 µl	JumpStart *Taq* Antibody (1.1 µg/µl; 7 µM)
5 µl	*Taq* DNA polymerase (5 units/µl; 0.25 µM)
10 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 12 PCR reactions using **0.8 µl** of the antibody:enzyme conjugate per reaction. The same reaction mix is prepared as in Section A, Step 1, with the difference in volume for the antibody:enzyme conjugate being made up with water. Please note the difference in volume from the JumpStart *Taq* Antibody diluted for immediate use.

Troubleshooting Guide

Problem	Solution
No reduction of nonspecific products is observed when using JumpStart *Taq* Antibody	Test the PCR system using a conventional hot start method. If both the JumpStart *Taq* PCR and the conventional hot start PCR yield multiple nonspecific products: • 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.[4]
	Take special precautions to avoid crossover contamination of PCR reactions with both specific and nonspecific PCR products, including primer-dimer artifacts.[5]

Troubleshooting Guide (Continued)

Problem	Solution
The JumpStart *Taq* PCR yields more nonspecific products than conventional hot start PCR	Titration of the JumpStart *Taq* 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 JumpStart *Taq* Antibody than recommended.
Yield of specific product is low using JumpStart Taq Antibody	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.
	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.
	DMSO up to 5% v/v is compatible with JumpStart antibody, but the use of other cosolvents may interfere with the antibody inactivation of the Taq polymerase.
The JumpStart Taq 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 JumpStart Taq Antibody may be necessary to alleviate the problem.

References

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