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ProductInformation

JumpStart™ Taq DNA Polymerase
With 10× Reaction Buffer containing $MgCl_2$

Product Code **D9307**
Technical Bulletin No. MB-395

TECHNICAL BULLETIN

Product Description

JumpStart™ Taq DNA polymerase (JumpStart Taq) is an optimized blend of Sigma's high-performance Taq DNA Polymerase and JumpStart™ Taq antibody. In JumpStart Taq DNA polymerase, the Taq activity is inactivated by combining the enzyme with JumpStart Taq antibody, a neutralizing monoclonal antibody to Taq DNA polymerase. The antibody inactivation provides a simple, efficient procedure for hot start PCR†. Hot start PCR can significantly improve the results of DNA amplifications by reducing the generation of nonspecific amplification products and primer-dimer artifacts. When used in PCR, JumpStart Taq DNA polymerase is inactive at low (room) temperature. When the temperature is raised above 70 °C in the first denaturation step of the cycling process, the complex dissociates and the polymerase becomes fully active.

Typical applications for JumpStart Taq 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.

The enzyme is supplied at 2.5 units/µl and comes with an optimized 10×-reaction buffer containing magnesium chloride.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Reagents Provided

- JumpStart Taq DNA polymerase, Product Code D6558
  2.5 Units/µl in 75 mM KCl, 15 mM Tris-HCl, pH 7.5, 50% glycerol, 0.05 mM EDTA, 0.5 mM DTT, 0.25% Tween® 20, and 0.25% IGEPAL® CA-630. Provided as 50, 250, or 1500 units.

- 10× PCR Buffer, Product Code P 2192
  100 mM Tris-HCl (pH 8.3 at 25 °C), 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin. Provided in 1.5 ml vials.

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

- 10 mM dATP, Product Code D6920
- 10 mM dCTP, Product Code D7045
- 10 mM dGTP, Product Code D7170
- 10 mM TTP, Product Code T7791
  or
- Deoxynucleotide mix, Product Code D7295 containing 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP
- PCR reagent water, Product Code W1754
- Mineral oil, Product Code M8662
- 0.2 and 0.5 ml PCR microcentrifuge tubes, thin-walled, Product Codes P3114 and P3364
- Thermal cycler
- Primers
- DNA to be amplified
- Chloroform, Product Code C7559 (optional)

Precautions and Disclaimer
JumpStart Taq DNA polymerase is for R&D use only. Not for drug, household or other uses. Refer to Material Safety Data Sheet.

Storage/Stability
Store at –20 °C.
JumpStart Taq DNA polymerase may be stored at 4 °C for up to 3 months and at –20 °C for up to six months. In the supplied storage buffer and at the supplied concentration, JumpStart Taq will not freeze at –20 °C. Freezing JumpStart Taq at storage below –20 °C is not recommended. Repeated freeze-thaw of JumpStart Taq may adversely affect its function.

Procedure
Note: The use of DMSO or formamide with JumpStart Taq DNA polymerase is not recommended due to interference with the enzyme-antibody complex. Other cosolvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart Taq antibody for the Taq polymerase and thereby compromise its effectiveness.

Preparation of PCR Master Mix and Thermal Cycling Parameters

Because the Taq polymerase is a magnesium ion-dependent enzyme, the optimal conditions for the concentration of Taq, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. ndividual component. This is especially true for the JumpStart Taq, cycling parameters, and the $MgCl_2$ concentration. It is recommended the enzyme and the $MgCl_2$ be titrated to determine the optimal efficiency.

To minimize tube-to-tube variation, preparation of a PCR master mix with JumpStart Taq 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 microcentrifuge tube in the following order:

Amount	Component	Final Concentration
- µl	Water	-
5 µl	10× PCR Buffer	1×
1 µl*	10 mM dATP	200 µM
1 µl*	10 mM dCTP	200 µM
1 µl*	10 mM dGTP	200 µM
1 µl*	10 mM TTP	200 µM
- µl	Primers	0.1-0.5 µM
1 µl	JumpStart Taq DNA Polymerase	0.05 units/µl
- µl	Template DNA (typically 10 ng)	200 pg/µl
50 µl	Total reaction volume	

*The individual nucleotides (1 µl of each 10 mM solution, 4 µl total) may be replaced by 1 µl of Deoxynucleotide Mix, Product Code D 7295.

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

3. Add 50 µl of mineral oil to the top of each tube to prevent evaporation (optional, depending on the model of thermal cycler).

4. Amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

   Typical cycling parameters:

Initial denaturation	94 °C	1 min
25-35 cycles:		
Denaturation	94 °C	30 sec
Annealing	55 °C to 68 °C	30 sec
Extension	72 °C	1 min (minimum)*
Final extension:	72 °C	1 min (minimum)*
Hold	4 °C	

* 1 minute minimum or 1 minute per kb expected amplicon.

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

JumpStart Taq DNA Polymerase Troubleshooting Guide

Problem	Suggestion
No reduction of nonspecific products is observed when using JumpStart Taq	Test the PCR system using a manual hot start method. The use of DMSO or formamide with JumpStart Taq is not recommended due to interference with the enzyme-antibody complex. Other cosolvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart Taq antibody for the Taq polymerase and thereby compromise its effectiveness.
Both the JumpStart Taq PCR and the manual 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.[1] Take special precautions to avoid crossover contamination of PCR reactions with both specific and nonspecific PCR products, including primer-dimer artifacts.[2]
The JumpStart Taq PCR yields more nonspecific products than manual hot start PCR	Titration of JumpStart Taq may be necessary to optimize the PCR reaction conditions, especially if the 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 than recommended.
The yield of specific product is low using JumpStart Taq.	Increase the reaction volume to 150 µl or more. 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. The use of DMSO or formamide with JumpStart Taq is not recommended due to interference with the enzyme-antibody complex.

References

1. Huang, L. M. and Jeang, K.-T. BioTechniques **16**:242-246 (1994)
2. Kwok, S. and Higuchi, R. Nature **339**:237-238 (1989)

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- Innis, M. A., et al. (Eds.), *PCR Strategies*, Academic Press, New York (1995). (Product Code Z36,445-2)
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†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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