

## Product Information

### JumpStart™ Taq DNA Polymerase

With 10× reaction buffer containing 15 mM MgCl<sub>2</sub>

Catalog Number **D9307**

Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

JumpStart™ Taq DNA Polymerase is an optimized blend of Sigma's high-performance Taq DNA Polymerase and JumpStart Taq antibody. The Taq DNA Polymerase 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, with an optimized 10× reaction buffer containing 15 mM MgCl<sub>2</sub>.

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, Catalog No. 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, stabilizers  
Provided as 50, 250, or 1500 units.

- 10× PCR Buffer, Catalog No. P2192  
100 mM Tris-HCl, pH 8.3, 500 mM KCl,  
15 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin.  
Provided in 1.5 ml vials.

### Reagents and equipment required but not provided

- 10 mM dATP sodium, Catalog Number D6920
- 10 mM dCTP sodium, Catalog Number D7045
- 10 mM dGTP sodium, Catalog Number D7170
- 10 mM TTP sodium, Catalog Number T7791  
or
- Deoxynucleotide mix, Catalog Number D7295 containing 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP sodium salts
- Chloroform, Catalog Number C7559 (optional)
- Mineral oil, Catalog Number M8662
- Water, PCR Reagent, Catalog Number W1754
- PurePak PCR microtubes, thin-walls, Catalog Numbers P3114 (0.2 ml) and P3364 (0.5 ml)
- Thermal cycler
- Primers
- DNA to be amplified

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store at –20 °C. 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 cycles may adversely affect its function.

## Procedure

**Note:** JumpStart *Taq* DNA polymerase has been shown to work effectively with up to 5% v/v DMSO. Other co-solvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart *Taq* antibody for the *Taq* DNA polymerase and thereby compromise its effectiveness.

### 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*, 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 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 microtubes in the following order:

Amount	Component	Final Concentration
x $\mu$ L	Water	
5 $\mu$ L	10 $\times$ PCR Buffer	1 $\times$
1 $\mu$ L*	10 mM dATP	200 $\mu$ M
1 $\mu$ L*	10 mM dCTP	200 $\mu$ M
1 $\mu$ L*	10 mM dGTP	200 $\mu$ M
1 $\mu$ L	10 mM TTP	200 $\mu$ M
y $\mu$ L	Primers	0.1-0.5 $\mu$ M
1 $\mu$ L	JumpStart <i>Taq</i> DNA Polymerase	0.05 units/ $\mu$ L
z $\mu$ L	Template DNA (typically 10 ng)	200 pg/ $\mu$ L
50 $\mu$ L	Total reaction volume	

\*The individual nucleotides (1  $\mu$ L of each 10 mM solution, 4  $\mu$ L total) may be replaced by 1  $\mu$ L of Deoxynucleotide Mix, Catalog Number D7295.

2. Mix gently and briefly centrifuge to collect all solution at the bottom of the tube.
3. Add 50  $\mu$ L 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
<b>25-35 cycles:</b>		
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.

### Troubleshooting Guide

Problem	Suggestion
No reduction of non-specific products is observed when using JumpStart <i>Taq</i>	<p>Test the PCR system using a manual hot start method.</p> <p>The use of more than 5% v/v DMSO or any other additive with JumpStart <i>Taq</i> is not recommended as it may interfere with the enzyme-antibody complex. Other co-solvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart <i>Taq</i> antibody for the <i>Taq</i> polymerase and thereby compromise its effectiveness.</p>
Both the JumpStart <i>Taq</i> PCR and the manual hot start PCR yield multiple non-specific products	<p>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.<sup>1</sup></p> <p>Take special precautions to avoid crossover contamination of PCR reactions with both specific and non-specific PCR products, including primer-dimer artifacts.<sup>2</sup></p>
The JumpStart <i>Taq</i> PCR yields more non-specific products than manual hot start PCR	Titration of JumpStart <i>Taq</i> 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 2 to 4-fold higher concentration of JumpStart <i>Taq</i> than recommended.
The yield of specific product is low using JumpStart <i>Taq</i> .	<p>Increase the reaction volume to 150 µL or more.</p> <p>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.</p> <p>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.</p> <p>The use of more than 5% v/v DMSO or any other additive with JumpStart <i>Taq</i> is not recommended as it may interfere with the enzyme-antibody complex.</p>

**References Cited**

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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