

## Product Information

### JumpStart™ REDTaq® DNA Polymerase

Catalog Number **D8187**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

JumpStart™ REDTaq® DNA Polymerase is Sigma's high performance Taq DNA Polymerase blended with JumpStart Taq antibody and an inert red dye tracer. Extensive testing with a variety of primers and templates indicates that the performance of JumpStart REDTaq DNA Polymerase is equivalent to, or better than, that of standard Taq polymerase.

- JumpStart REDTaq DNA Polymerase is the ideal enzyme for high throughput and/or multiplex PCR applications.
- Reactions are prepared the same way as standard PCR mixtures requiring no additional reaction preparation steps or protocol changes.
- The hot start mechanism using JumpStart Taq antibody prevents non-specific product formation and allows assembled PCR reactions to be placed at room temperature up to 2 hours without compromising performance.
- Red tracer means quick recognition of reactions to which enzyme has been added, as well as visual confirmation of complete mixing.
- The enzyme is provided at 1 unit/ $\mu\text{L}$  for more accurate volume measurement and less waste.
- The enzyme formulation allows aliquots (5-10  $\mu\text{L}$ ) from the PCR to be directly loaded onto an agarose gel without addition of loading buffers.
- The red tracer serves as a tracking dye co-migrating at the same rate as a 125 bp fragment in a 1% agarose gel.

Since the red tracer has no effect on the amplification process, a sample can be easily re-amplified such as in "nested PCR". The presence of the dye also has no effect on automated DNA sequencing; ligase mediated ligations, exonucleolytic PCR product digestion, and transformation. Though exceptions may exist, the dye is generally inert in restriction enzyme digestions. If necessary, the dye can be removed from the amplicon by routine purification methodologies.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 min at  $74\text{ }^{\circ}\text{C}$ .

### Reagents provided

- JumpStart REDTaq DNA Polymerase, Catalog Number D0563  
1 unit/ $\mu\text{L}$  in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% TWEEN® 20, inert dye, 50% glycerol. Provided as 50, 250 or 2,500 units (10  $\times$  250 units)
- 10 $\times$  PCR Buffer, Catalog Number P2192  
100 mM Tris-HCl, pH 8.3 at  $25\text{ }^{\circ}\text{C}$ , 500 mM KCl, 15 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin. Provided in 1.5 ml vials.

### Reagents and equipment required but not provided

- Primers
- DNA to be amplified
- 10 mM dATP, Catalog Number D6920
- 10 mM dCTP, Catalog Number D7045
- 10 mM dGTP, Catalog Number D7170
- 10 mM TTP, Catalog Number T7791  
or
- Deoxynucleotide mix, Catalog Number D7295, containing 10 mM each dATP, dCTP, dGTP, and TTP
- Water, PCR reagent, Catalog Number W1754
- Mineral oil, Catalog Number M8662 (optional)
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Catalog Numbers P3114 and P3364
- Thermal cycler

### 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  $-20\text{ }^{\circ}\text{C}$ .

JumpStart REDTaq DNA Polymerase, as supplied, will not freeze at  $-20\text{ }^{\circ}\text{C}$ . It is not recommended to freeze this product at storage temperatures below  $-20\text{ }^{\circ}\text{C}$ . Repeated freeze/thaw cycles may adversely affect its function.

### Procedure

**Note:** The use of DMSO or formamide with JumpStart REDTaq DNA Polymerase is not recommended due to interference 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 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  $\text{MgCl}_2$  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 JumpStart REDTaq, cycling parameters, and the  $\text{MgCl}_2$  concentration. It is recommended the enzyme and the  $\text{MgCl}_2$  be titrated to determine the optimal efficiency.

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

1. Add the following reagents to a 0.2 ml or 0.5 ml PCR tube.

Volume	Reagent	Final Concentration
5 $\mu\text{L}$	10 $\times$ PCR Buffer	1 $\times$
1 $\mu\text{L}^*$	10 mM dATP	200 $\mu\text{M}$
1 $\mu\text{L}^*$	10 mM dCTP	200 $\mu\text{M}$
1 $\mu\text{L}^*$	10 mM dGTP	200 $\mu\text{M}$
1 $\mu\text{L}^*$	10 mM TTP	200 $\mu\text{M}$
- $\mu\text{L}$	Primers	0.1-0.5 $\mu\text{M}$
2.5 $\mu\text{L}$	JumpStart REDTaq DNA Polymerase	0.05 units/ $\mu\text{L}$
- $\mu\text{L}$	Template DNA (typically 10 ng)	200 pg/ $\mu\text{L}$
	Water	-
50 $\mu\text{L}$	Total reaction volume	

\*The individual nucleotides (1  $\mu\text{L}$  of each 10 mM solution, 4  $\mu\text{L}$  total) may be replaced by 1  $\mu\text{L}$  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  $\mu\text{L}$  of mineral oil to the top of each tube to prevent evaporation (optional, depending on 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 $^{\circ}\text{C}$ for 1 min
<b>25-35 cycles:</b>	
Denaturation	94 $^{\circ}\text{C}$ for 30 sec
Annealing	55 $^{\circ}\text{C}$ to 68 $^{\circ}\text{C}$ for 30 sec
Extension	72 $^{\circ}\text{C}$ for 1 min (minimum)*
Final extension:	72 $^{\circ}\text{C}$ for 1 min (minimum)*
Hold	4 $^{\circ}\text{C}$

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

5. The amplified DNA can be evaluated by loading 5-10  $\mu\text{L}$  of the PCR reaction directly onto agarose gel. It is not necessary to add a separate loading buffer/tracking dye.

Note: A minimum of 1.5 units of JumpStart REDTaq DNA polymerase must be added per 50  $\mu\text{l}$  reaction to ensure enough glycerol is present for direct gel loading. The red tracer comigrates with 125 bp fragment in a 1% agarose gel.

### Troubleshooting Guide

Problem	Suggestion
No reduction of non-specific products is observed when using JumpStart REDTaq DNA Polymerase.	Test the PCR system using a manual hot start method.
	The use of DMSO or formamide with JumpStart REDTaq DNA Polymerase is not recommended due to interference 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 Taq antibody for the Taq polymerase and thereby compromise its effectiveness.
Both the JumpStart REDTaq PCR and the manual hot start PCR yield multiple nonspecific products.	Raise the annealing temperature in 2-3 $^{\circ}\text{C}$ 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>
	Take special precautions to avoid crossover contamination of PCR reactions with both specific and nonspecific PCR products, including primer-dimer artifacts. <sup>2</sup>
The JumpStart REDTaq PCR yields more non-specific products than conventional hot start PCR.	Titration of JumpStart REDTaq may be necessary to achieve the same degree of improvement as with a conventional hot start. This is especially true if 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 REDTaq than recommended.
The yield of specific product is low using JumpStart REDTaq.	Increase the reaction volume to 150 $\mu\text{L}$ 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 $^{\circ}\text{C}$ to overcome denaturation difficulties.
	The use of DMSO or formamide with JumpStart REDTaq 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)

### General References

- Griffin, H. G., and Griffin, A. M., (Eds.), *PCR Technology: Current Innovations*, CRC Press, 1994, Catalog Number Z357499.
- Innis, M. A., et al., (Eds.), *PCR Strategies*, Academic Press, New York (1995), Catalog Number Z364452.
- Innis, M., et al., (Eds.), *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990), Catalog Number P8177.
- Newton, C.R., (Ed.), *PCR: Essential Data*, John Wiley & Sons, New York (1995).
- Sambrook, J. F., et al., *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000), Catalog Number M8265.

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