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ProductInformation

JumpStart™ REDTaq™ DNA Polymerase

Product Code **D 8187**
Technical Bulletin No. MB-750

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 or better than that of standard Taq polymerase.

- The ideal enzyme for high throughput and/or multiplex PCR† applications.
- Reactions using JumpStart REDTaq 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 one unit per microliter for more accurate volume measurement and less waste.
- The enzyme formulation allows aliquots (5-10 µl) from the PCR to be directly loaded onto an agarose gel without addition of loading buffers.
- The red tracer serves as a tracking dye comigrating 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 °C.

Reagents Provided

- JumpStart REDTaq DNA Polymerase, Product Code D0563
  1 unit/µl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 0.5% Igepal® CA-630, inert dye, 50% glycerol. Provided as 50, 250 or 2,500 units (10×250 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.)

- Primers
- DNA to be amplified
- 10 mM dATP, Product Code D 6920
- 10 mM dCTP, Product Code D 7045
- 10 mM dGTP, Product Code D 7170
- 10 mM TTP, Product Code T 7791

  or
- Deoxynucleotide mix, Product Code D 7295 containing 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
- Thermal cycler

Precautions and Disclaimer
JumpStart REDTaq 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.
As supplied storage buffer and at the supplied concentration, JumpStart REDTaq will not freeze at –20 °C. Freezing JumpStart REDTaq at storage below –20 °C is not recommended. Repeated freeze-thaw of JumpStart REDTaq may adversely affect its function.

Procedure
Note: The use of DMSO or formamide with JumpStart REDTaq 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. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the JumpStart REDTaq, 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 REDTaq 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 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
2.5 μl	JumpStart REDTaq DNA Polymerase	0.05 units/μl
- μl	Template DNA (typically 10 ng)	200 pg/μl
	Water	-
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 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 for 1 min
25-35 cycles:	
Denaturation	94 °C for 30 sec
Annealing	55 °C to 68 °C for 30 sec
Extension	72 °C for 1 min (minimum)*
Final extension:	72 °C for 1 min (minimum)*
Hold	4 °C

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

5. The amplified DNA can be evaluated by loading 5-10 µl 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 µl reaction to ensure enough glycerol is present for direct gel loading. The red tracer comigrates with 125 bp fragment in a 1% agarose gel.

JumpStart REDTaq DNA Polymerase Troubleshooting Guide

Problem	Suggestion
No reduction of nonspecific products is observed when using JumpStart REDTaq.	Test the PCR system using a manual hot start method.
	The use of DMSO or formamide with JumpStart REDTaq 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 REDTaq 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 REDTaq PCR yields more nonspecific 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 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 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. (Product Code Z35,749-9)
- Innis, M. A., et al., (Eds.), *PCR Strategies*, Academic Press, New York (1995). (Product Code Z36,445-2)
- Innis, M., et al., (Eds.), *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990). (Product Code P 8177)
- Newton, C.R., (Ed.), *PCR: Essential Data*, John Wiley & Sons, New York (1995). (Product Code Z36,491-6)
- Sambrook, J. F., et al., *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000). (Product Code M 8265)

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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