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Product Information

JumpStart™ REDTaq® ReadyMix™ Reaction Mix

Catalog Number **P0982**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

JumpStart REDTaq ReadyMix combines the performance and convenience benefits of Sigma's REDTaq Genomic DNA polymerase and the advantages of JumpStart *Taq* Antibody for hot start[1] PCR in an easy-to-use reaction mixture. This is the ideal solution for performing high-throughput PCR, combining quick setup time with the ability to load samples immediately after PCR onto agarose gels. This ready-to-use mixture of JumpStart REDTaq Genomic DNA polymerase, 99% pure deoxynucleotides and reaction buffer is provided in a 2× concentrate for ease-of-use. Add 25 µL of the 2× mix, DNA template, primers and water. At room temperature, the JumpStart *Taq* antibody inactivates the REDTaq Genomic DNA polymerase. 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. After amplification, the sample is loaded directly onto an agarose gel. The unique inert red dye acts as a tracer, migrating slightly faster than bromophenol blue. There are no special preparation steps or protocol changes required. JumpStart REDTaq DNA polymerase offers the same high quality performance as regular *Taq* DNA polymerase.

- The ideal ReadyMix for high throughput and/or multiplex PCR applications.
- For a typical PCR reaction, mix 25 µL of JumpStart REDTaq ReadyMix with 25 µL of a mixture containing template DNA, primers, and water. Reaction volume can be scaled if desired.
- A hot start mechanism using JumpStart *Taq* antibody, which prevents non-specific product formation, allows assembled PCR reactions to be placed at room temperature for up to 2 hours without compromising the performance.
- When performing a large number of PCR reactions, JumpStart REDTaq ReadyMix can save a significant amount of preparation time, reduce the risk of contamination from multiple pipetting steps, and provide consistent batch-to-batch and reaction-to-reaction performance.
- The red tracer means quick recognition of reactions to which enzyme has been added as well as visual confirmation of complete mixing.
- 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 with a 125 bp fragment in a 1% agarose gel.

Because the red tracer has no effect on the amplification process, a sample can be easily re-amplified as in "nested PCR". The presence of the dye also has no effect on automated DNA sequencing, ligation, exonucleolytic PCR product digestion, and transformation. Although 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 methods.

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

Reagent

Supplied as 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 4 mM $MgCl_2$, 0.002% gelatin, 0.4 mM each dNTP (dATP, dCTP, dGTP, TTP), inert dye, stabilizers, 0.1 unit/µL *Taq* DNA Polymerase, JumpStart *Taq* antibody. Available as 20, 100 and 800 reactions (default reaction volume is 50 µL).

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
Product can be stored at 2-8 °C for up to 6 months so there is no waiting for the reaction components to thaw. It can also be stored at –20 °C, long term. There was no detectable loss of performance after 10 freeze-thaw cycles.

Materials and Reagents Required but not Provided
- PCR Reagent water, Catalog Number W1754
- Primers
- DNA template
- Thermal cycler
- 0.2 and 0.5 mL PCR microcentrifuge tubes, thin-walled, Catalog Numbers P3114 and P3364
- Mineral Oil, Catalog Number M8662 (optional)
- Betaine, Catalog Number B0300 (optional)
- DMSO, Catalog Number D8418 (optional)

Procedure
Optimal concentrations of template DNA, $MgCl_2$, KCl and PCR adjuncts, as well as pH, are often target specific. If further optimization is needed for specific template and primers, additional components ($MgCl_2$, dNTPs, KCl, betaine, etc.) can be added to the template/primer mixture, although this is not required for most applications. The following procedure serves as a reference.

Note: DMSO up to 5% (v/v) is compatible with the system and does not interfere with PCR. However, 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.

1. Add the following reagents to a 0.2 ml or 0.5 ml thin-walled microcentrifuge tube.

Volume	Reagent	Final Concentration
25 µL	JumpStart REDTaq ReadyMix	1×
--- µL	Forward primer (20 µM)	0.4 µM
--- µL	Reverse primer (20 µM)	0.4 µM
--- µL	Template DNA	1-200 ng
q.s.	Water	
50 µL	Total volume	

Note: A template-primer mix is recommended when performing multiple reactions.

2. Mix gently by vortexing and briefly centrifuge to collect all components 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. Optimum cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality.

Typical cycling parameters for 0.2–2 kb fragments:

Initial denaturation	94 °C	2 min
30-35 cycles:		
Denaturation	94 °C	30 sec
Annealing	55 °C to 68 °C	30 sec
Extension	72 °C	2 min
Final extension	72 °C	5 min
Hold	4 °C	

5. The amplified DNA can be evaluated by loading 5-10 µL of the PCR reaction directly onto an agarose gel. It is not necessary to add a separate loading buffer/tracking dye.

References

1. Dieffenbach, C., and Dveksler, G., (Eds.), *PCR Primer: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2003.
2. Rees, W. A., et al., *Biochemistry*, **32**, 137-144 (1993).
3. Don, R. H., et al., *Nucleic Acids Res.*, **19,** 4008 (1991).
4. Huang, L. M., and Jeang, K.-T., *Biotechniques*, **16,** 242-246 (1994).
5. Kwok, S., and Higuchi, R., *Nature*, **339**:237-238 (1989).

Troubleshooting Guide

Problem	Possible Cause	Solution
No PCR product is observed	A PCR component is missing or degraded.	A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.
	Too few cycles were performed.	Increase the number of cycles (3-5 additional cycles at a time).
	The annealing temperature is too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primer to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%.
	There is not enough template.	After increasing the number of cycles has shown no success, repeat the reaction with a higher concentration of template.
	The template is of poor quality.	Evaluate the template integrity by gel electrophoresis. It may be necessary to repurify template using methods that minimize shearing and nicking.
	The denaturation temperature is too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time is too long or too short.	Optimize the denaturation time by increasing or decreasing it in 10 second increments.
	The extension time is too short.	Increase the extension time in 2 minute increments.
	Target template is complex.	In most cases, inherently complex targets are due to unusually high GC content and/or secondary structure. Betaine has been reported to help amplification of high GC content templates at a concentration of 0.8-1.3 M.[2]
There are multiple or smeared products	The annealing temperature is too low.	Increase the annealing temperature in increments of 2-3 °C.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primers to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primers with a GC content of 45-60%.
	Touchdown PCR may be required.	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications. Touchdown PCR involves using an annealing/extension temperature that is higher than the T_M of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer T_M for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.[3]
	Too many cycles were performed.	The nonspecific bands may be eliminated by reducing the number of cycles.
	The template concentration is too high.	Reduce the concentration of the template in the PCR reaction.
	The template concentration is too low.	Add additional template in 50 ng increments for genomic DNA or 1-2 ng for viral DNA.
There is no reduction of nonspecific PCR bands when using the JumpStart enzyme.	The antibody affinity may be reduced by reaction components or conditions.	Some co-solvents, solutes (e.g., salts) and pH extremes may reduce the affinity of the JumpStart *Taq* antibody for the polymerase and thereby compromise its effectiveness. Check your reaction mixture and conditions and/or check your system with a manual hot start method.
	Primers were not designed appropriately.	Check your system with a manual hot start method. If the results are similar, raise the annealing temperature in 2-3 °C increments to improve the specificity of binding. If raising the temperature reduces the yield of the specific product with only a small reduction of side reaction products, it may be necessary to redesign the primers.[4]

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
There is no reduction of nonspecific PCR bands when using the JumpStart enzyme. (continued)	There was crossover contamination of specific and/or nonspecific PCR products.	Take special precautions to avoid crossover contamination of PCR reactions, including primer-dimer artifacts.[5]
The yield of specific product is low.	Too few cycles were performed.	Increase the cycle number in 3-5 cycle increments.
	A co-solvent is required.	Add dimethyl sulfoxide (5%) or 0.8-1.3 M betaine final concentration.
	PCR priming opportunities may be low due to reaction conditions or primer design.	Modify the reaction conditions by increasing the denaturation temperature to 95 °C, increase extension times in 2 minute increments, increase $MgCl_2$ and dNTP concentrations, etc. Redesign PCR primers.
The finished PCR reaction does not sink in the well of the agarose gel.	There is too little REDTaq in the reaction mix; the mix was diluted.	Add loading buffer to the reaction aliquot.

Related Products

Reagents

- Lambda DNA *Hind* III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kit, HSRT100 (100 reactions)

Equipment

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

Trademarks

The following trademarks and registered trademarks are accurate to the best of our knowledge at the time of printing. Please consult individual manufacturers and other sources for specific information.

Sigma-Aldrich Co. LLC. – REDTaq®, JumpStart™ and ReadyMix™

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