3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

REDAccuTaq® LA DNA Polymerase

Catalog Number **D4812**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
REDAccuTaq LA DNA Polymerase offers all the performance of AccuTaq LA DNA Polymerase for long and accurate PCR† with the added benefits of easy visualization and direct loading. It is an optimized blend of our high performance *Taq* DNA polymerase, a small amount of a polymerase with the 3'→5' exonuclease activity necessary for proofreading, and a unique inert red dye that acts as a tracer and loading buffer.

PCR amplifications from 0.25 to 20 kb are achievable. REDAccuTaq LA DNA Polymerase is ideal for amplifying complex DNA templates, such as human genomic DNA, and its fidelity is 6.5 times greater than *Taq* DNA polymerase alone. This high fidelity makes it the enzyme of choice when performing amplifications in which low error frequency is critical, such as RT-PCR and cloning.

Reactions using REDAccuTaq LA DNA Polymerase and its optimized 10× buffer are prepared the same way as standard PCR mixtures, requiring no additional reaction preparation steps or protocol changes. The red tracer provides 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/µL for more accurate volume measurement and less waste. This formulation also allows aliquots (5-10 µL) from the PCR to be directly loaded onto an agarose gel without addition of electrophoresis 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.

If necessary, the dye can be removed from the amplicon by routine purification methodologies. The presence of the dye has no effect on automated DNA sequencing, ligase mediated ligations, exonucleolytic PCR product digestion or transformation. Though exceptions may exist, the dye is generally inert in restriction enzyme digestions.

Reagents Provided
- REDAccuTaq LA DNA Polymerase Mix, Catalog Number D4937 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, 50% glycerol, inert dye. Provided as 50, 250, and 2500 units.
- AccuTaq LA 10× Buffer, Catalog Number B0174, 0.5 ml vial, 500 mM Tris-HCl, 150 mM ammonium sulfate (pH 9.3, adjusted with KOH), 25 mM $MgCl_2$, 1% TWEEN 20. Provided as 1 vial/50 units, 2 vials/250 unit, and 12 vials/2,500 units

Materials and reagents required but not provided
- Deoxynucleotide Mix, Catalog Number D7295, 10 mM each dATP, dCTP, dGTP, and TTP
- Water, PCR Reagent, Catalog Number W1754
- Mineral Oil, Catalog Number M8662 (optional)
- Primers
- DNA to be amplified
- Dedicated pipettes
- PCR pipette tips
- 0.2 or 0.5 ml thin-walled PCR microcentrifuge tubes
- Thermal cycler
- Betaine solution, 5 M, Catalog Number B0300 (optional)
- DMSO, Catalog Number D8418 (optional)

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.

Preparation Instructions

Reaction Optimization

Reliable amplification of long DNA sequences requires: 1) effective denaturation of DNA template, 2) adequate extension times to produce large products and 3) protection of target DNA from damage by depurination. Effective denaturation is accomplished by the use of higher temperatures for shorter periods of time or by the use of co-solvents, such as dimethyl sulfoxide. Addition of DMSO in the reaction at a final concentration between 1 to 4% may increase yield and improve reliability of the system with some complex PCR targets. Betaine (0.8-1.3 M) has been reported to improve the amplification of DNA by reducing the formation of secondary structure in GC rich regions.[1]

Thermal Cycler

A Perkin-Elmer GeneAmp 2400 cycler was used to develop cycling parameters. Other types of thermal cyclers can also be used, but may require further optimization of cycling parameters.

Primer design

Primers are usually 21 to 34 bases in length and are designed to have a GC content of 45-50%. Optimally, the melting temperatures of the forward and reverse primers should be within 3 °C of each other and the Tm of the primers should be between 65-72 °C.[2] Primers should not have any internal base-pairing sequences (i.e., potential hairpins) or any significant length of complementary regions between the two PCR primers. It is sometimes helpful to design primers with a final CC, GG, CG, or GC on the 3-prime end of the primers in order to increase priming efficiency.[3]

Template

High quality and adequate length of the template are essential for reliable amplification of larger fragments. Extreme care must be taken in the preparation and handling of the DNA target for long PCR. Nicked or damaged DNA can serve as a potential priming site resulting in high background. Avoid freezing, or, alternatively, freeze only once to minimize damage. The condition of the target DNA is critical. Depurination during cycling is minimized by use of buffers with a pH greater than 9.0 at 25 °C.

Magnesium concentration

Optimization of magnesium concentration may be necessary. Generally magnesium concentrations should be between 1 and 5 mM.

Cycle Conditions

Extension temperature should be limited to 68 °C for optimal performance. Temperatures greater than 68 ºC may result in a reduced amount or no product. For targets greater than 20-kb, extension times should be greater than 20 minutes. Primer annealing and product extension can also be combined into one step if primers are designed to have a Tm between 65-68 °C. The use of auto-extension is advisable to reduce artifacts. Cycle denaturation times should be kept short. For example, the initial DNA denaturation may be accomplished by a 30-second incubation at 96 °C.

Buffer preparation

The AccuTaq LA 10× Buffer is at a relatively high pH, and magnesium may precipitate as magnesium hydroxide [$Mg(OH)_2$]. Before use, thaw the buffer at room temperature, then vortex to redissolve any precipitated $Mg(OH)_2$. Alternatively, warm the buffer at 37 °C for 3-5 minutes, then vortex.

Storage/Stability

Store REDAccuTaq LA DNA Polymerase and AccuTaq LA 10× Buffer at –20 °C.

Amplification Procedure

The optimal conditions for the concentration of REDAccuTaq LA DNA Polymerase, 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.

1. Add the following reagents to a thin-walled 200 µl or 500 µL PCR microcentrifuge tube:

Volume	Reagent	Final Concentration
5 µL	AccuTaq LA 10× Buffer	1X
2.5 µL	dNTP Mix D7295	500 µM
- µL	Template DNA*	4 ng/µL
- µL	Forward Primer	0.1-1 µM
- µL	Reverse Primer	0.1-1 µM
- µL	Water	----
2.5 µL	REDAccuTaq LA DNA Polymerase Mix	0.05 units/µL
50 µL Total Volume		

- Typically ≥200 ng template DNA is necessary for amplification of more complex genomes.

2. Mix gently and briefly centrifuge to collect all components to 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. The amplification parameters should be optimized for individual primers, template, and thermal cycler. Suggested cycling parameters based on in-house amplification of lambda DNA and a 20 kb fragment of human β-globin gene cluster:

Initial denaturation	98 °C 30 sec
For cycle 1-15:	
Denaturation	94 °C 5-15 sec
Annealing	65 °C 20 sec
Extension	68 °C 20 min
For cycle 16-30:	
Denaturation	94 °C 5-15 sec
Annealing	65 °C 20 sec
Extension	68 °C 20 min+15 sec/cycle
Final extension:	68 °C 20 min
Hold	4 °C

5. Evaluate the amplified DNA by agarose gel electrophoresis and subsequent ethidium bromide staining.[4]

Notes:

a. When amplifying templates 20 kb or greater, a 15 second auto-extension is suggested for cycles 16-30. Some thermal cyclers may not have this auto-extension function; increasing the extension time by 1-4 minute increments is recommended.
b. When amplifying fragments less than 20 kb, the extension time can be reduced according to the fragment size. Normally, one minute extension time will be sufficient for 1 kb fragment.

References

1. Rees, W.A. *et al*., *Biochemistry*, **32**, 137-144 (1993).
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3. Lowe, T., *et al*., A computer program for selection of oligonucleotide primers for polymerase chain reaction. *Nucleic Acids Res.* **18**, 1757-1761 (1990).
4. Sambrook, J, *et al., Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000), Catalog Number M8265.
5. Don, R. H., *et al.,* "Touchdown" PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.* **19,** 4008 (1991).
6. Barnes, W. M., PCR amplification of up to 35-kb DNA with high fidelity and high yield from λ bacteriophage templates. *Proc. Natl. Acad. Sci. USA* **91**, 2216-2220 (1994).
7. Cheng, S., *et al.,* Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc. Natl. Acad. Sci. USA* **91**, 5695-5699 (1994).
8. Roux, K. H., Optimization and troubleshooting in PCR. *PCR Methods Appl.* **4,** 5185-5194 (1995).

Troubleshooting Guide

Problem	Possible Causes	Solution
No PCR product is observed	A PCR component may be missing or degraded.	A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.
	There may be too few cycles performed.	Increase the number of cycles (3-5 additional cycles at a time).
	The annealing temperature may be too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers may not be 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 may not be enough template.	After increasing the number of cycles has shown no success, repeat the reaction with a higher concentration of template.
	The template may be of poor quality.	Evaluate the template integrity by agarose gel electrophoresis. It may be necessary to repurify template using methods that minimize shearing and nicking.
	The denaturation temperature may be too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time may be too long or too short.	Optimize the denaturation time by increasing or decreasing it in 10 second increments.
	The extension time may be too short.	Increase the extension time in 2 minute increments, especially for long templates.
	The reaction may not have enough enzyme.	0.05 units/μL is sufficient for most applications. It is recommended that the cycling parameters be optimized before the enzyme concentration is increased. In rare cases, the yields can be improved by increasing the enzyme concentration. However, if the enzyme amount is above 0.10 units/μL, higher background levels may be seen.
	Mg^{++} levels may be suboptimal.	This is unlikely if the 10X reaction buffer (with $MgCl_2$) is used and the deoxynucleotides do not exceed a concentration of 0.6 mM each (as deoxynucleotide triphosphates can bind Mg^{++}). Typically, $MgCl_2$ is optimized between 1 to 5 mM. Also, EDTA present in the sample at greater than 5 mM will reduce the effective concentration of magnesium.
	Deoxynucleotide amounts are too low.	This is unlikely if the final concentration of each deoxynucleotide is 0.5 mM. This concentration of dNTPs is suitable for a wide range of applications. If the dNTPs are being prepared in the laboratory, be sure that the final concentration of each deoxynucleotide is 0.5 mM. If the concentration of dNTPs is increased, the Mg^{++} concentration will need to be increased proportionately.
	Target template is difficult.	In most cases, inherently difficult 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. In some cases, the addition of 1-4% DMSO may help.
	The PCR product floated out of the well during direct loading into the gel.	Use the recommended amount of REDAccuTaq LA DNA Polymerase (2.5 μL per 50 μL reaction) to provide enough glycerol for proper loading of PCR product directly into the gel.

Troubleshooting Guide (Continued)

Problem	Possible Causes	Solution
Multiple products	There may be too many cycles performed.	By reducing the cycle number, the nonspecific bands may be eliminated.
	The annealing temperature may be too low.	Increase the annealing/extension temperature in increments of 2-3 °C.
	The primers may not be 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 needed.	"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 Tm of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer Tm for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.[5]
Products are smeared	Too many cycles may have been performed.	Reduce the cycle number in 3-5 cycle increments.
	The denaturation temperature may be too low.	Increase the denaturation temperature in 1 °C increments.
	The extension time may be too long.	Decrease the extension time in 1-2 minute increments
	Touchdown PCR may be needed.	See recommendations under "Multiple Products" for procedure.
	There may be too much enzyme in the reaction mix.	0.05 units/μL is sufficient for most applications. However, this concentration may be too high for some applications. It is recommended the cycling parameters be optimized first, as described above, then, reduce the enzyme concentration to 0.5-0.2X.
	Magnesium concentration may be too high.	The $MgCl_2$ concentration should be optimized. Typically, the concentration of $MgCl_2$ is optimal between 1 and 5 mM. If the concentration of the dNTPs is 0.5 mM, it is very unlikely that the magnesium concentration is too high.
	The template concentration may be too high.	Reduce the concentration of the template in the PCR reaction.
Product is wrong size	The primers may not be designed optimally.	See recommendations under "Multiple Products".
	The extension time may be too short.	Increase the extension times in 2 minute increments or use touchdown PCR.
Faint product	The template concentration may be too low.	Add additional template in 50 ng increments for genomic DNA or 1-2 ng for viral DNA.
	There may be too few cycles performed.	Increase the cycle number in 3-5 cycle increments
	The extension time may be too short.	Increase the extension times in 2 minute increments
	A co-solvent may be required	Add dimethyl sulfoxide (1-4%) or 0.8-1.3 M betaine final concentration.

Related Products

Reagents

- PCR Optimization Kit II, Catalog Number OPT2
- Lambda DNA Hind III Digest, Catalog Number D9780
- Enhanced Avian HS RT-PCR kits, Catalog Numbers HSRT20 (20 reactions) and HSRT100 (100 reactions). This kit combines enhanced avian reverse transcriptase and JumpStart AccuTaq LA for hot start PCR.
- BlueView™ Nucleic Acid Stain, Catalog Numbers T8935 and T9060.

Equipment

- PCR Multiwell Plates, 96-well, Catalog Number Z374903; 384-well, Catalog Number Z374911
- PCR Microtubes, 0.2 ml with attached caps, Catalog Number Z374873; 0.2 ml strip tubes with strip caps, Catalog Number Z374962
- Sealing accessories for PCR vessels
  - Pierceable cap strips, Catalog Number Z374954, caps in strips of eight, can be used with 0.2 ml PCR strip and 96-well plates. Pkg of 120 strips (960 caps).
  - Micro Mats, Catalog Number Z374938, molded to fit standard 96-well plates
- PCR Workstation, 120V, Catalog Number Z376213; 240V, Catalog Number Z376221

Books

- *PCR: A Practical Approach* Catalog Number P7186
- *PCR 3: PCR In Situ Hybridization* Catalog Number Z378399
- *PCR In Bioanalysis: Methods in Molecular Biology*, Vol. 92 Catalog Number Z379603
- *PCR Primer: A Laboratory Manual* Catalog Number Z364118
- *PCR Protocols: A Guide to Methods and Applications,* Catalog Number P8177
- *PCR Protocols for Emerging Infectious Diseases* Catalog Number Z369918
- *PCR Sequencing Protocols* Catalog Number Z373818
- *PCR Strategies,* Catalog Number Z364452
- *PCR Technology, Current Innovations* Catalog Number Z357499
- *Quantitation of mRNA by Polymerase Chain Reaction,* Catalog Number Z371947

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