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Product Information

AccuTaq™ LA DNA Polymerase

Catalog Number **D8045**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

AccuTaq LA DNA Polymerase combines Sigma's high quality Taq DNA polymerase with a small amount of a polymerase with the 3'→5' exonuclease activity necessary for proofreading. The result is an enzyme mix that amplifies genomic targets in excess of 20 kb. Using a less complex template, such as bacterial genomic or viral DNA, amplifications of up to 20 kb or 40 kb, respectively, have been achieved. The fidelity of AccuTaq LA is 6.5 times greater than Taq DNA polymerase alone.

The Polymerase Chain Reaction (PCR) using *Taq* DNA polymerase is generally limited to amplifications up to 5 kb. This is due in part because Taq DNA polymerase has no 3'→5' exonuclease or "proofreading" activity, which means periodic mis-incorporations are not repaired. After a mis-incorporation has taken place, the enzyme will either continue to incorporate nucleotides, causing a processive mistake, or a terminal event will occur and elongation will be arrested. Long and Accurate (LA) PCR combines a highly processive thermostable polymerase with a second thermostable polymerase that exhibits a 3'→5' exonucleolytic activity. This blend increases the length of amplification products by using the proofreading polymerase to repair terminal mis-incorporations. This repair allows the polymerase to resume elongating the growing DNA strand. In addition, this blend creates products with mostly blunt ends as a result of the proofreading activity.

Reagents

- AccuTaq LA DNA Polymerase, Catalog No. D5553. 5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, 0.5% IGEPAL® CA-630, 50% glycerol
  Provided in quantities of 125, 500, and 1500 units
- AccuTaq LA 10× Buffer, Catalog No. B0174
  0.5 ml vial; 500 mM Tris-HCl, 150 mM ammonium sulfate (pH 9.3, adjusted with NH_4OH), 25 mM $MgCl_2$, 1% TWEEN® 20. Provided as 3 vials/125 units, 3 vials/500 unit, and 9 vials/1,500 units
- Dimethyl sulfoxide (DMSO), Catalog Number D8418. Provided as 1.5 ml

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
- 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 No. B0300 (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.

Storage/Stability

Store AccuTaq LA DNA Polymerase and the10× Buffer at –20 °C. Store DMSO at room temperature. Melting frozen DMSO at ~30 °C will not affect performance.

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 of 1-4% 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 DNA Cycler 480 has been 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 50%. Optimally, the melting temperatures (T_m) of the forward and reverse primers should be within 3 °C of each other and between 60-72 °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 1-5 mM.

Cycle Conditions
Extension temperature should be limited to 68 °C for optimal performance. Temperatures greater than 68 °C may result in a reduced amount of, 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 T_m 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
AccuTaq LA 10x Buffer is at a relatively high pH, and magnesium may precipitate as $Mg(OH)_2$. 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.

Procedures

A. Amplification Procedure for Genomic DNA
The optimal conditions for the concentration of AccuTaq 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	1×
2.5 µL	dNTP Mix (10 mM each)	500 µM
5 µL	Template DNA* (40 ng/uL)	4 ng/µL
1 µL	DMSO	2%
1 µL	Forward Primer (20 pmole/µL)	400 nM
1 µL	Reverse Primer (20 pmole/µL)	400 nM
34 µL	Water, PCR Reagent	----
0.5 µL	AccuTaq LA DNA Polymerase	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 λ-globin gene cluster fragments from human genomic DNA):

Initial denaturation	98 °C	30 sec.
For cycles 1-30		
Denaturation	94 °C	5-15 sec.
Annealing	65 °C	20 sec.[a]
Extension	68 °C	20 min.[b]
Final extension	68 °C	10 min.

Notes:

a. Oligonucleotides were between 21 bases (high G+C content) and 34 bases (high A+T content) in length. Melting temperatures of oligonucleotides used for amplification of genomic DNA were 62-70 °C. This was determined using the algorithm based upon nearest neighbor analysis of Rychlik and Rhoads.[2]

b. 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. If it is not available, increasing the extension time by 1-4 minute increments is recommended.

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

B. Amplification Procedure for Lambda DNA.

The optimal conditions for the concentration of Taq 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	1×
2.5 µL	dNTP Mix (10 mM each)	500 µM
3 µL	Template DNA (2.5 ng/µL)	0.15 ng/µL
3 µL	Forward Primer (10 pmole/µL)	600 nM
3 µL	Reverse Primer (10 pmole/µL)	600 nM
33 µL	Water	----
0.5 µL	AccuTaq LA DNA Polymerase Mix	0.05 units/µL
50 µL	**Total Volume**	

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:

Initial denaturation	98 °C	30 sec
For cycles 1-30		
Denaturation	94 °C	5-15 sec
Annealing	65 °C	20 sec[c]
Extension	68 °C	20 min[d]
Final extension	68 °C	10 min

Notes:

c. Oligonucleotides were between 21 bases (high G+C content) and 34 bases (high A+T content) in length. Melting temperatures of oligonucleotides used for amplification of genomic DNA were 62-70 °C. This was determined using the algorithm based upon nearest neighbor analysis of Rychlik and Rhoads.[2]

d. 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. If it not available, increasing the extension time by 1-4 minute increments is recommended.

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

References

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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.	0.5 µL (2.5 units) 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 1 µL (5 units), higher background levels may be seen
	Mg^{++} levels may be too low.	This is unlikely if the 10× reaction buffer (provided) is used and the deoxynucleotides do not exceed a concentration of 0.6 mM each. However, if the concentration of EDTA in the sample is greater than 5 mM, this can reduce the effective concentration of magnesium..

Troubleshooting Guide (continued)

Problem	Possible Causes	Solution
No PCR product is observed (continued)	Deoxynucleotides 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.
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 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.[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.	0.5 µL (2.5 units) 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 magnesium concentration has been optimized in the supplied 10× reaction buffer. 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" for procedure.
	The extension time may be too short.	Increase the extension times in 2-minute increments or use Touchdown PCR.
Faint Product	There may not be enough template.	Add additional template in 50 ng increments for genomic or 1-2 ng for viral.
	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 DMSO (1-4%), or 0.8-1.3 M betaine, final concentration

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