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Product Information

KlenTaq® LA DNA Polymerase Mix

Catalog Number **D5062**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

KlenTaq® LA DNA Polymerase Mix is a specially blended enzyme mix containing KlenTaq-1 DNA polymerase (a 5′-exo-minus, N-terminal deletion of Taq DNA polymerase) and a small amount of a proofreading DNA polymerase. This blending of KlenTaq-1 and a proofreading polymerase increases the fidelity, yield, and the length of the amplified product. KlenTaq-1 is more efficient and more processive than either native Taq DNA polymerase or other N-terminal deletions of Taq. This means the same degree of amplifications can be achieved with fewer cycles. KlenTaq LA has a broad magnesium optimum, so it is typically unnecessary to optimize the magnesium concentration in the reaction mixtures. It has fidelity four times greater than that seen in standard Taq DNA polymerase. KlenTaq LA is ideal for DNA amplifications 0.5–5 kb in length on genomic DNA and up to 10 kb on less complex templates.

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

Reagents Provided

- KlenTaq LA DNA Polymerase Mix,
  Catalog No. D5187
  5 units/µL in 50% glycerol, 40 mM Tris-HCl (pH 7.5), 50 mM KCl, 25 mM $(NH4)_2SO_4$, 0.1 mM EDTA, 5.0 mM 2-mercaptoethanol, 0.25% Thesit® (polyoxyethylene 9 lauryl ether). A proofreading DNA polymerase is a minor component of KlenTaq LA polymerase mix.
  Provided as 125 units, 500 units, or 1,500 units
- 10× Buffer for KlenTaq LA, Catalog No. B6178,
  400 mM Tricine-KOH (pH 9.2 at 25 °C), 150 mM KOAc, 35 mM $Mg(OAc)_2$, 750 µg/ml bovine serum albumin.
  Provided as 1.5 ml vial, 1 vial per 125 units or 500 units of KlenTaq LA polymerase, 3 vials per 1,500 units of KlenTaq LA polymerase

Equipment and Reagents Required, Not Provided

- Deoxynucleotide Mix, Catalog No. D7295
  10 mM dATP, 10 mM dCTP, 10 mM dGTP and 10 mM TTP
- Water, PCR Reagent, Catalog No. W1754
- DNA template to be amplified
- Primers
- Mineral Oil, Catalog No. M8662 (optional)
- Dedicated pipettes
- PCR pipette tips
- 0.5 ml or 0.2 mL thin-walled PCR tubes, Catalog Nos. P3114 and P3364
- Dimethyl sulfoxide (DMSO), PCR Reagent, Catalog No. D9170 (optional)
- Betaine, 5 M solution, Catalog No. B0300 (optional)
- 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

Store at –20 °C. Storage in "frost-free" freezers is not recommended.

Preparation Instructions

Reaction Optimization

The optimal conditions for amplification will vary based on the template DNA, primers, experimental protocols, tubes, and thermal cyclers. Reliable amplification of long DNA sequences requires:

1) effective denaturation of DNA template
2) adequate extension times to produce large products
3) protection of target DNA from damage by depurination

Depurination during cycling is minimized by the use of a buffer with a pH >9.0 at 25 °C. 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 1–4% 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 secondary structure in GC-rich regions.[1]

Thermal Cycler

Perkin-Elmer DNA Cyclers 480, 2400, and 9600 have 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

Primer design is critical for successful long PCR. Primers are usually 21–34 bases in length and designed to have a GC content of 45–50%. In general, primers should have a T_m of at least 70 °C if a 68 °C annealing/extension step is used. Optimally, the melting temperatures of the forward and reverse primers should be within 3 °C of each other and the T_m of the primers should be between 65–72 °C. 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 may helpful to design primers with a final CC, GG, CG, or GC on the 3′ end of the primers in order to increase priming efficiency.[2]

Template

KlenTaq LA can tolerate a wide range of template quality. However, as the length of the target increases, the quality of the target becomes more important. High quality and adequate length of the template are essential for reliable amplification of larger fragments. The number of full-length unnicked targets decreases as the length of the target increases. Nicked or damaged DNA can serve as a potential priming site resulting in high background. Extreme care must be taken in the preparation and handling of the DNA target for long PCR. Avoid freezing or alternatively, freeze only once to minimize damage.

Magnesium concentration

Optimization of magnesium concentration is not necessary with KlenTaq LA. KlenTaq has been found to be insensitive to magnesium concentration.[3]

Cycle Conditions

Extension temperature should be limited to 68 °C for optimal performance. Temperatures >68 °C may result in a reduced amount or no product. Primer annealing and product extension can also be combined into one step if primers are designed to have a T_m between 65–72 °C. Cycles for denaturation parameters should be kept short to minimize both damage to the template and inactivation of the enzyme. The half-life of the enzyme is markedly decreased at temperatures >96 °C.

Touchdown PCR

"Touchdown" PCR has been shown to significantly improve the specificity of many PCR reactions in a wide variety of applications.[4] Briefly, touchdown PCR uses an annealing/extension temperature that is several degrees (typically 3–10 °C) higher than the T_m of the primers during the initial cycles (typically 5–10). The annealing/extension temperature is then reduced to the primer T_m for the remaining cycles.

Procedure

1. Add the following reagents to a thin-walled 0.2 or 0.5 mL PCR tube:

Volume	Reagent	Final Concentration
5.0 µL	10× PCR Buffer for KlenTaq LA	1×
1.0 µL	dNTP Mix (10 mM each)	200 µM
– µL	Forward Primer	0.1–1 µM
– µL	Reverse Primer	0.1–1 µM
– µL	Template DNA	1–10 ng
1 µL	KlenTaq LA Polymerase	0.1 unit/µL
q.s.	Water	–
50 µL	Total Volume	

Note: Less DNA template is required if amplifying plasmid DNA (typically 1–5 ng). If amplifying from a genomic "pool", more DNA template may be necessary.

2. Mix gently by vortex 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 if not using a thermal cycler with a heated lid.

4. The 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.

Common cycling parameters based on length of target

	<5 kb	**5–10 kb**
Initial denaturation	94 °C for 1 min	95 °C for 1 min
For Cycles 1 to 25–35		
Denaturation	94 °C for 30 sec	94 °C for 30 sec
Annealing/ Extension	68 °C for 3–5 min	68 °C for 6–10 min
Final extension (optional)	68 °C for 3 min (May reduce background)	68 °C for 10 min
Hold	4 °C	4 °C

5. The amplified DNA can be evaluated by 0.8–1.0% agarose gel electrophoresis and subsequent ethidium bromide staining.[5]

Notes:

For most applications, a two-step cycling program is recommended over a three-step cycling program. A two-step cycling program involves denaturation at T_1, followed by annealing and extension at T_2. A three-step cycling program has separate temperatures for denaturation, annealing, and extension. Three-step PCR is more flexible and is necessary when the T_m of the primers is <70 °C. Below is a sample of parameters for a three-step PCR program based on amplification of a 10 kb lambda fragment.

Three Step PCR for 10 kb targets

Initial Denaturation	95 °C for 1 min
For Cycles 1 to 25/35	
Denaturation	94°C for 30 sec
Annealing	55 °C for 15 sec
Extension	68 °C for 10 min
Final extension (optional)	68 °C for 10 min
Hold	4 °C

The number of cycles will depend on the abundance of target DNA. For multiple copy genes or medium to high abundance cDNAs, 25 cycles is sufficient. For single or low copy number genes or rare cDNAs, 30-35 cycles is recommended.

Denaturation times should be as short as possible. *Long initial denaturation times (>1 minute) are detrimental and unnecessary*. In some cases, better results are seen with a 15 second cycle initial denaturation time at 95 °C. Exposure of DNA to high temperatures causes some depurination of single-stranded DNA during denaturation, which eventually leads to strand scission. For cycling denaturation, one should keep in mind that although KlenTaq-1 is stable at elevated temperatures of 95 °C, high temperatures can lead to a gradual loss of enzymatic activity. Minimizing denaturation time is particularly important in experiments with very long templates where total cycling time can exceed 12 hours.

References

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2. Lowe, T. et al., A computer program for selection of oligonucleotide primers for polymerase chain reaction. *Nucleic Acids Res.* **18,** 1757-1761 (1990).
3. Barnes, W. M., PCR amplification of up to 35-kb DNA with high fidelity and high yield from bacteriophage templates. *Proc. Natl. Acad. Sci. USA* **91,** 2216-2220 (1994).
4. Don, R.H. et al., 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res*. **19,** 4008 (1991).
5. Sambrook, J, et al., *Molecular Cloning: A Laboratory Manual,* Third Edition, Cold Spring Harbor Laboratory Press, New York (2000). Catalog No. M8265
6. 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).
7. Chou, Q., Prevention of pre-PCR mispriming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res*. **20,** 1717-1723 (1992).
8. D'Aquila, R.T., et al., Maximizing sensitivity and specificity of PCR by preamplification heating. *Nucleic Acids Res*. **19,** 3749 (1991).
9. Roux, K. H., Optimization and troubleshooting in PCR. *PCR Methods Appl.* **4,** 5185-5194 (1995).
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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 <22 nucleotides long, try to lengthen the primer to 25–30 nucleotides. If the primer has a GC content of <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 the template using methods that minimize shearing and nicking.
	The denaturation temperature may be too high or 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 1 minute increments, especially for long templates.
	The reaction may not have enough enzyme.	One μL (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 concentration is above 2 μL (10 units), higher background levels may be seen.
	Magnesium 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 0.2 mM each. KlenTaq-1 has a broad magnesium optimum. However, if the concentration of EDTA in the sample is >5 mM, this can reduce the effective concentration of magnesium.
	Deoxynucleotides are too low.	This is unlikely if the final concentration of each deoxynucleotide is 0.2 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 0.2 mM. If the concentration of dNTPs is increased, the magnesium 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 2–5% DMSO may help.

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 <22 nucleotides long, try to lengthen the primers to 25–30 nucleotides. If the primer has a GC content of <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.
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 under "Multiple Products" for touchdown PCR procedure.
	There may too much enzyme in the reaction mix.	One µL (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 0.5–0.2×.
	Magnesium concentration may be too high.	KlenTaq-1 has a broad magnesium optimum and the magnesium concentration has been optimized in the supplied 10× reaction buffer. If the 10× reaction buffer is being used and the final concentration of each of the dNTPs is 0.2 mM, it is very unlikely the magnesium concentration is too high.
	The template concentration may be too high.	Reduce the concentration of the template in the PCR reaction.
Contamination	Contamination usually results in extra bands or smearing. It is recommended to include a water control (in place of the DNA template) with every PCR reaction to determine if the reagents used in the PCR reaction are contaminated with a template from a previous reaction.	
	When performing PCR directly on phage plaques or bacterial colonies, failure to isolate single plaques or colonies can produce multiple bands.	

Related Products

- PCR Optimization Kit II, Catalog No. OPT2
- Lambda DNA *Hin*d III Digest, Catalog No. D9780
- BlueView™ Nucleic Acid Stain, Catalog Nos. T8935 and T9060. When used to prepare agarose gel and as running buffer, BlueView instantly stains nucleic acids
- Enhanced Avian HS RT-PCR kits, Catalog Nos. HSRT20 (20 reactions), HSRT100 (100 reactions). Combines two powerful techniques to convert mRNA into cDNA and subsequently to amplify the cDNA. Offers an enhanced ability to transcribe through difficult secondary structure at elevated temperatures (up to 65 °C)

Equipment

- PCR microtube, 0.2 mL, Catalog No. Z374873
- PCR microtube, 0.65 mL, Catalog No. Z374881
- PCR strip tubes, 8 × 0.2 mL, with strip caps, Catalog No. Z374962
- PCR multiwell plates, 96-well, Catalog No. Z374903
- Micro mats for 96 well plates, Catalog No. Z374938
- PCR multiwell plates, 384-well, Catalog No. Z374911
- Sealing accessory for PCR vessels, Pierceable cap strips, Catalog No. Z374954, caps in strips of eight, the center of each can be pierced with a hypodermic needle for quick sample removal without generating aerosols or other sources of cross-contamination. Caps can be used with 0.2 mL PCR strip and 96-well plates.
- PCR Workstation, 120 V, Catalog No. Z376213
- PCR Workstation, 240 V, Catalog No. Z376221

Books

- *PCR: A Practical Approach*, M. J. McPherson, P. Quirke and G. R. Taylor, Eds., IRL Press, Oxford, England, 1991, Catalog No. P7186
- *PCR 3: PCR In Situ Hybridization*, C. S. Herrington and J. J. O'Leary, Eds., IRL Press at Oxford University Press, Inc., Oxford, England, 1997, Catalog No. Z378399
- *PCR In Bioanalysis: Methods in Molecular Biology*, Vol. 92, S. J. Smeltzer, Ed., Humana Press, Totowa, NJ, 1998, Catalog No. Z379603
- *PCR Primer: A Laboratory Manual*, C. Dieffenbach and G. S. Dvekster, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1995, Catalog No. Z364118
- *PCR Protocols: A Guide to Methods and Applications*, M. A. Innis, et al., Eds., Academic Press, San Diego, CA, 1990, Catalog No. P8177
- *PCR Protocols for Emerging Infectious Diseases*, D. H. Pershing, Ed., American Society of Microbiology, Washington, DC, 1996, Catalog No. Z369918
- *PCR Sequencing Protocols*, R. Rapley, Humana Press, Totowa, NJ, 1996, Catalog No. Z373818
- *PCR Strategies*, Michael A. Innis, David H. Gelfand, and John J. Sninsky, Eds., Academic Press, San Diego, CA, 1995, Catalog No. Z364452
- *PCR Technology, Current Innovations*, H. G. Griffin and A. M. Griffin, Ed., CRC Press, Boca Raton, FL, 1994. Catalog No. Z357499

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