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

RED KlenTaq® DV ReadyMix™

Catalog Number **D1816**

TECHNICAL BULLETIN

Product Description

Sigma's RED KlenTaq® DV ReadyMix™ combines the performance benefits of KlenTaq DNA polymerase with the convenience of a ReadyMix. This ready-to-use mixture of KlenTaq-1 DNA polymerase (a 5′-exo-minus, N-terminal deletion of Taq DNA polymerase), 99% pure deoxynucleotides, reaction buffer, an inert red dye, and a small amount of a proofreading DNA polymerase is provided in a 2x concentrate. KlenTaq-1 is more efficient and more processive than either native Taq DNA polymerase or other N-terminal deletions of Taq. This means a higher amount of product is generated with the same number of cycles than when using Taq DNA polymerase. KlenTaq-1 has a broad magnesium optimum, so it is typically unnecessary to optimize the magnesium concentration in the reaction mixtures.

KlenTaq DV is an excellent polymerase for applications requiring high PCR yields from less complex, thoroughly purified templates. KlenTaq DV will amplify a 3 kb amplicon from lambda DNA or 1-1.5 kb using human genomic DNA as a template. The ReadyMix is ideal for high throughput applications.

The inert red dye allows for easy confirmation of enzyme addition and visualization of complete mixing during setup. After the PCR reaction, the PCR product can be loaded directly onto an agarose gel. There is no need to add a loading buffer/tracking dye prior to electrophoresis. The red dye migrates at approximately the same rate as a 125 base pair fragment. Because the red dye has no effect on the amplification process, a sample can be easily re-amplified such as in “nested PCR”.

RED KlenTaq DV ReadyMix is provided as a 2 x concentrate, which saves preparation time and reduces the risk of contamination from multiple pipetting steps. RED KlenTaq DV ReadyMix provides consistent reaction-to-reaction performance.

- Better efficiency and yield than Taq-based formulations, due to the higher processivity of KlenTaq-1
- Magnesium optimization is unnecessary, since KlenTaq works over a broad magnesium range
- Excellent amplification up to 3 kb for less complex templates (plasmids and lambda DNA)
- Immediate confirmation that not only has the enzyme been added, but that proper mixing has occurred. An aliquot can be taken directly from the reaction and loaded onto an agarose gel for electrophoresis.
- For a typical PCR reaction, mix 25 µL of RED KlenTaq DV ReadyMix with 25 µL of a mixture containing template DNA, primers, and water. Reaction volumes can be scaled down, if desired.
- When performing large numbers of PCR reactions, RED KlenTaq DV ReadyMix can save a significant amount of preparation time, reduce the risk of contamination from multiple pipetting steps, and provide consistent performance

Reagents Provided

- RED KlenTaq DV ReadyMix, Catalog Number D1816
  0.16 units/µL REDKlenTaq DNA polymerase, 100 mM Tris-HCl, pH 9.1, 33 mM ammonium sulfate, 7 mM $MgCl_2$, 300 µg/ml BSA, 0.4 mM dNTP mix (dATP, dCTP, dGTP, TTP), 2 µM EDTA, 0.2 mM 2-mercaptoethanol, stabilizers, inert dye, and a small amount of proofreading enzyme
  Provided as 20 and 100 reactions, 50 µL per reaction.

Reagents and equipment required but not provided

- Template to be amplified
- Primers
- Thermal cycler
- Dedicated pipettes
- PCR pipette tips
- 0.2 or 0.5 ml PCR microcentrifuge tubes, thin-walled, Catalog Numbers P3114 and P3364

Precautions and Disclaimer
RED KlenTaq DV ReadyMix is for R&D use only. Not for drug, household or other uses. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. Refer to Material Safety Data Sheet.

Storage/Stability
Store RED KlenTaq DV ReadyMix below –20 °C.

Preparation Instructions
Reaction Optimization
The optimal conditions for amplification will vary based on the template DNA, primers, experimental protocols, tubes, and thermal cyclers. KlenTaq varies from Taq DNA polymerase in that the optimal extension is at a lower temperature (68 °C vs 72 °C). In addition, Klentaq DV is inefficient in amplifying targets greater than 3 kb in length.

Addition of DMSO in the reaction at a final concentration between 1 to 5% 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]

Magnesium concentration
Optimization of magnesium concentration is not necessary because KlenTaq has been found to be insensitive to magnesium concentration.[2]

Procedure
The optimal conditions for template DNA, primers, and cycling parameters will depend on the system being utilized.

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

Volume	Reagent	Final Concentration
25 µL	RED KlenTaq DV ReadyMix (2x concen-trate)	4 units KlenTaq DNA polymerase, 50 mM Tris-HCl, pH 9.1, 16.5 mM $(NH_4)_2SO_4$, 3.5 mM $MgCl_2$, 200 µM each dNTP
1 µL	Forward Primer	0.1-1.0 µM (15-30 bases in length)
1 µL	Reverse Primer	0.1-1.0 µM (15-30 bases in length)
X µL	Template DNA	1-10 ng
Y µL	Water	-
50 µL	Total Volume	

2. Mix gently, then 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.

   Typical cycling parameters:

Initial denaturation	94 °C	1 min
For cycles 1-30:		
Denaturation	94 °C	15 sec
Annealing/ Extension	68 °C	3 min
Final extension:	68 °C *	10 min
Hold	4 °C	

   * We have noted lower yields with 72 °C extension temperatures.

5. The amplified DNA can be loaded directly onto an agarose gel after the PCR process. There is no need to add a loading buffer/tracking dye prior to electrophoresis. The red tracer migrates at approximately the same rate as a 125 base pair fragment, slightly faster than bromophenol blue. Because the red tracer has no effect on the amplification process, a sample can be easily re-amplified such as in “nested PCR”. If desired, the red chromophore can be removed by normal purification methods such as ethanol precipitation, solid phase cleanup systems, phenol extraction, etc.

Note: 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 less than 70 °C.

References

1. Rees, W.A., *et al*., Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry***, 32**, 137-144 (1993).
2. 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).
3. Don, R.H. *et al*., ‘Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.*, **19**, 4008 (1991).

AF,CR,PHC,MAM 09/10-1

Label License Statement

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser’s own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Troubleshooting Guide

Problem	Causes	Solution
No PCR product is observed.	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 22 nucleotides long, try to lengthen the primer to 25-30 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 low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	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.
	Target amplicon is too long.	KlenTaq DV is formulated for amplifications of ≤3 kb using lambda DNA template.
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 22 nucleotides long, try to lengthen the primers to 25-30 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.[3]
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.