LongerAmp *Taq* DNA Polymerase

Catalog: RK20680
Size: 1,000 U / 2,500U
Concentration: 2,500 U/ml
Components:

LongerAmp *Taq* DNA Polymerase (2,500 U/ml)	RM20312
5X LongerAmp *Taq* Reaction Buffer	RM20140

Product Description

LongerAmp *Taq* DNA Polymerase is a unique blend of *Taq* klenow and Deep Ocean DNA Polymerases. The 3´→5´ exonuclease activity of Deep Ocean DNA Polymerase increases the fidelity and robust amplification of *Taq* klenow DNA Polymerase. LongerAmp *Taq* DNA Polymerase offers two fold higher fidelity than *Taq* klenow DNA Polymerase alone. A wide range of PCR products can be generated, up to 30 kb from lambda DNA or from human genomic DNA.

Unit Definition:
One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Storage Conditions:
10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween 20, 0.5% IGEPAL CA-630, 50% Glycerol, pH7.4 @ 25°C

Storage Temperature: -20°C

Reaction Conditions: 1X LongerAmp *Taq* Reaction Buffer

1X LongerAmp *Taq* Reaction Buffer:
60 mM Tris-SO_4, 20 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 3% Glycerol, 0.06% IGEPAL CA-630, 0.05% Tween 20, pH9.1 @ 25°C

Application Features:
Long Range PCR; Colony PCR

Heat Inactivation: No
5' - 3' Exonuclease: No
3' - 5' Exonuclease: Yes
Strand Displacement: +
Resulting Ends: Mix of Blunt and Single-base 3´ Overhangs
Notes: 5'→3' flap endonuclease destroys displaced strand.

Instructions

Reaction setup: We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

Component	25 µl	50 µl	Final Conc.
Nuclease-free water	to 25 µl	to 50 µl	
5X LongerAmp *Taq* Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.75 µl	1.5 µl	300 µM
10 µM Forward Primer	1 µl	2 µl	0.4 µM (0.05–1 µM)
10 µM Reverse Primer	1 µl	2 µl	0.4 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
LongerAmp *Taq* DNA Polymerase	1 µl	2 µl	5 units/50 µl PCR

Notes: Gently mix the reaction. Avoid pipetting samples containing target DNA when amplicons above 20 kb are desired. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes from ice to a PCR machine with the block preheated to 94°C and begin thermocycling. A PCR program was recommended.

Thermocycling conditions for a routine PCR:

STEP	STEP	TIME
Initial Denaturation	94°C	3 minutes
30 Cycles	98°C	5-10seconds
	55-60°C(optional)	20-30seconds
	65-68°C	50 seconds/kb
Final Extension	65-68°C	10 minutes
Hold	4-10°C	

General Guidelines:

1. Template:

The quality of the DNA template is essential for long-range PCR amplification. Recommended amounts of DNA template for a 50 μl reaction are as follows:

DNA	up to 15 kb	above 15 kb
genomic	1 ng–500 ng	10 ng–1 μg
plasmid or viral	1 pg–1 ng	10 pg–10 ng

Successful amplification above 20 kb largely depends on the quality of DNA templates and the primer sequences.

2. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. For amplicons larger than 20 kb, it is desirable to have primers with GC content above 50%, matched Tm above 60°C and primers at least 24 nucleotides in length. The final concentration of each primer in a PCR reaction may be 0.05–1 μM, typically 0.1–0.5 μM.

3. Mg++ and additives:

Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with LongerAmp *Taq* DNA Polymerase. The final Mg++ concentration in 1X LongerAmp *Taq* Reaction Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.5 or 1.0 mM increments using $MgSO_4$.

Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Deoxynucleotides:

The recommended final concentration of dNTPs for long-range PCR is 300 μM of each deoxynucleotide.

5. LongerAmp *Taq* DNA Polymerase concentration:

We generally recommend using LongerAmp *Taq* DNA Polymerase at a concentration of 100 units/ml (5 units/50 μl reaction). However, the optimal concentration of LongerAmp *Taq* DNA Polymerase may vary in specialized applications.

6. Denaturation:

An initial denaturation of 3 minutes at 94°C is sufficient for most amplicons from pure DNA templates. For difficult templates or longer amplicon, a higher initial denaturation at 98°C is recommended prior to PCR cycling to fully denature the template.

During thermocycling a 5–10 second denaturation at 98°C is recommended.

7. Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–65°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m. When primers with annealing temperatures above 60°C are used, a 2-step PCR protocol is possible (see #10).

8. Extension:

The recommended extension temperature is 65-68°C. Extension times are generally 50 seconds per kb. A final extension of 10 minutes at 65-68°C is recommended. Notice: 72°C was not suitable for this product.

9. Cycle number:

Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. 2-step PCR:

When primers with annealing temperatures above 60°C are used, a 2-step thermocycling protocol is possible. Moreover, When PCR product is longer than 5kb, a 2-step PCR protocol is recommended especially for human or *E.coli* genomic.

Thermocycling conditions for a routine 2-step PCR:

Temperature	Time	Cycles
94°C	3min	1
98°C 65-68°C	5-10s 50s/kb	30 cycles
65-68°C 4-10°C	10 min ∞	1

11. PCR product:

The majority of the PCR products generated using LongerAmp *Taq* DNA Polymerase contain dA overhangs at the 3´–end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

12. Optimization:

Denature temperature, extension temperature, and different time for every step were optimized for special and difficult amplicon. This optimization was very important for successful results.