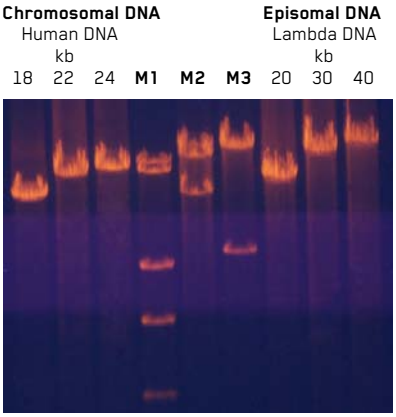
Amplus DNA Polymerase

Thermus sp. DNA Polymerase
Pyrococcus sp. DNA Polymerase
Polymerase Enhancing Factor

Cat. No.	Size
E2900-01	100 units
E2900-02	500 units

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 min at 74°C. The reaction conditions are: 50 mM Tris-HCl (pH 9.0 at 25°C), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^{3}H]dTTP), 10 µg activated calf thymus DNA and 0.1 mg/ml BSA in a final volume of 50 µl.

Storage Conditions: Store at -20°C.



PCR amplification using EURx Amplus DNA Polymerase.
Lanes 18, 22, 24 kb: PCR amplification reactions, using respectively 2, 1.75 or 1.5 U of Amplus DNA Polymerase and 250-500 ng of human genomic DNA as a template for 35 cycles in 50 µl reaction volume (3, 4 and 8 µl of 50 µl reaction volume were analyzed on a 0.5% [w/v] agarose gel).
Marker lanes:
Lambda DNA (GenBank Acc.No. J02459)
Lane M1: Lambda DNA digested with HindIII, 23, 9.5, 6.7, 4.4 kb.
Lane M2: Lambda DNA digested with KpnI, 30, 17 kb.
Lane M3: Lambda DNA digested with ApaI, 38.5, 10 kb.
Lanes 20, 30, 40 kb: PCR amplification reactions, using 2 U of Amplus DNA Polymerase and 5-20 ng of lambda DNA as a template for 25 cycles in 50 µl reaction volume (2, 2.5 and 4 µl of 50 µl reaction volume were analyzed on a 0.5% [w/v] agarose gel).
Preparation of large sized template DNA:
Human genomic DNA was isolated from HeLa cells using EURx GeneMatrix Tissue DNA Purification Kit (Cat. No. E3550).

Mixture of thermostable DNA polymerases, a polymerase-enhancing factor and a unique polymerase buffer enabling long-range PCR amplification of genomic targets longer than 25 kb and episomal targets up to 40 kb.

Description:

- Amplus DNA Polymerase is a modified and optimized thermostable enzymes blend containing *Thermus* sp. DNA polymerase, *Pyrococcus* sp. DNA polymerase and a polymerase-enhancing factor.
- Ultrapure, recombinant enzymes are used to prepare Amplus DNA Polymerase.
- A unique composition of the amplification buffer enables effective buffering of pH at high temperatures and allows to achieve robust yield from long genomic targets.
- The polymerase amplifies efficiently large genomic DNA fragments from 3 kb to over 25 kb and episomal fragments up to 40 kb. It also provides reliable amplification of smaller fragments (as small as 0.1 kb).
- The enzymes blend is especially suitable for eukaryotic genome amplification, cloning and analysis.
- Ideal for genome mapping and sequencing by greatly simplifying contig assembly from large amplification products.
- Enables characterization of cloned sequences in lambda phages and cosmids.
- Improves PCR results with critical templates, such as containing GC-rich regions.
- Amplus DNA Polymerase exhibits 3' → 5' proofreading activity, resulting in considerably higher PCR fidelity and processivity than possible with unmodified *Taq* DNA polymerase (1).
- Maintains 5' → 3' exonuclease activity.
- Adds extra A at the 3' ends.

Storage Buffer:

20 mM Tris-HCl (pH 8.2 at 22°C), 100 mM KCl, 0.5% Tween 20, 0.5% Igepal CA-630, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol.

10 x Reaction Buffer:

10 x Amplus Buffer:
The buffer contains 26 mM $MgSO_4$ and is optimized for use with 0.5 mM of each dNTP.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. *Cline, J., Braham, J. and Hogrefe, H. (1996) Nucleic Acids Res. 24, 3546.*

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Amplus DNA Polymerase

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Amplus Buffer, containing 26 mM $MgSO_4$.	5 µl	1x
dNTP mix (5mM each)	5 µl	0.5 mM each dNTP
Upstream primer	Variable	0.3 µM
Downstream primer	Variable	0.3 µM
Amplus DNA Polymerase, 5U/µl **(a)** genomic targets up to 20 kb and episomal targets up to 40 kb **(b)** genomic targets over 20 kb	 0.4 µl 0.3 - 0.35 µl	 2 U 1.5 - 1.7 U
Template DNA	Variable	< 0.5 µg/µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Thermal Cycling Conditions for Products between 0.1 and 5 kb in Size:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	92-94˚C	2 min	1
Denaturation	92-94˚C	10 s	25-35
Annealing	60-68˚C	30 s	
Extension	68˚C	1 min/1 kb	
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Thermal Cycling Conditions for Products Larger Than 5 kb in Size:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	92-93˚C	2 min	1
Denaturation	92-93˚C	10 s	10
Annealing	60-68˚C	30 s	
Extension	68˚C	1 min/1 kb	
Denaturation	92-93˚C	10 s	15-25
Annealing	60-68˚C	30 s	
Extension	68˚C	**1 min/1 kb +20 s per additional cycle**	
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. This is especially important for magnesium solutions, because they form a concentration gradient when frozen.
2. Prepare reaction mixes on ice, mix well.
3. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. For Amplus DNA Polymerase-based PCR, the standard concentration of $MgSO_4$ is 2.6 mM (as provided by the 1 x Amplus Buffer). In most cases this concentration will produce satisfactory results. Should the reaction require inceased Mg^{2+} concentrations, use the supplied 25 mM $MgSO_4$ solution for adjustment.
5. 2 U of Amplus DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. In case of **genomic targets over 20 kb** use 1.5-1.75 U instead of 2 U per 50 µl amplification reaction. In some cases (especially for very long genomic targets over 18-20 kb) it may be necessary to optimize the enzyme concentration to achieve its best performance.
6. The recommended concentration of primers is 0.3 µM. This concentration enables to achieve satisfying yield while not compromising amplification specificity from genomic targets. Increasing the concentration to 0.4-0.5 µM may increase yield but lead to reduced specificity.
7. For long range PCR use: 100-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, 1-50 ng of phage DNA or 1-20 ng of plasmid DNA.
8. The quality of the template influences dramatically the performance of PCR. Ensure that a template DNA is of sufficiently high quality. Use only high-molecular-weight DNA, when amplifying long PCR targets (over 20-50 kb, depending on the amplicon length). Template DNA should be prepared carefully, applying gentle methods that do not shear the template in order to receive high molecular weight DNA of high purity.
9. Complex genomic DNA should be stored at 2-8˚C and should not have been frozen. Avoid vortexing the genomic DNA. Avoid freeze-thaw steps.
10. Use only thin-walled 0.2 ml tubes when performing long PCR amplification.
11. In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as GC-rich sequences and very long targets over 30 kb, additives such as DMSO may be included to improve amplification.

Notes:

1. Annealing temperature should be optimized for each primer set based on the primer T_m. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5˚C below T_m.
2. Typical primers for long PCR amplification reactions have a length of 22-34 bp and should have annealing temperatures above 60˚C to enhance reaction specificity.
3. When amplifying long PCR products, keep denaturation steps as short as possible and denaturation temperature as low as possible **For genomic DNA exceeding 15 kb in length, a denaturation temperature of max. 92˚C is strongly recommended** (genomic DNA is much more liable to breaks than episomal DNA, especially at high denaturation temperatures). Sometimes fragments with high GC-content need higher denaturation temperatures, but keep in mind that the yield increases when denaturation temperature / duration is decreased.
4. For PCR products between 5 and 10 kb in length, an elongation of the extension step (+20 s in each additional cycle, starting from the 11th cycle) is optional (but recommended) but allows better yields due to loss of processivity of the enzymes blend. For PCR products longer than 10 kb the elongation of extension step is necessary to achieve satisfying results.

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