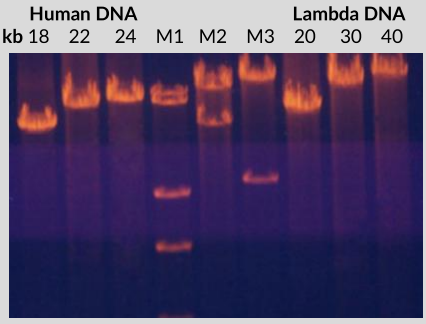
EURx® MOLECULAR BIOLOGY PRODUCTS

tiAmplus DNA Polymerase HOT START

Cat. No.	size
EK2930-01	100 units
EK2930-02	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 minutes 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 tiAmplus DNA Polymerase.
Lanes 18, 22, 24 kb: PCR amplification reactions, using respectively 1.75, 1.5 or 1.5 U of tiAmplus 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 the 0.5% agarose gel).
Lane M1: Lambda DNA digested with HindIII.
Lane M2: Lambda DNA digested with KpnI.
Lane M3: Lambda DNA digested with ApaI.
Lanes 20, 30, 40 kb: PCR amplification reactions, using 1.5 U of tiAmplus DNA Polymerase and 3-10 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 the 0.5% agarose gel). Human genomic DNA was isolated from HeLa cells using EURx GeneMatrix Tissue DNA Purification Kit.

References:

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

Mixture of "hot start" thermophilic 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:

- tiAmplus DNA Polymerase is a new generation "hot start" enzyme blend that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during normal cycling conditions.
- tiAmplus DNA Polymerase is a modified and optimized thermostable enzyme blend containing *Thermus sp.* DNA polymerase, *Pyrococcus sp.* DNA polymerase and the polymerase-enhancing factor.
- Ultrapure, recombinant enzymes are used to prepare tiAmplus 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 enzyme 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.
- tiAmplus DNA Polymerase exhibits the 3'→5' proofreading activity, resulting in considerably higher PCR fidelity and processivity than possible with unmodified Taq DNA polymerase (1).
- Maintains the 5'→3' exonuclease activity.
- Adds extra A at the 3' ends.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

10 x Reaction Buffer:

10 x Amplus Buffer:
The buffer contains 29 mM $MgSO_4$ and is optimized for use with Eurx dNTP mix.

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.

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Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Amplus Buffer contains 29 mM $MgSO_4$	5 µl	1x
dNTP mix (5 mM each)	5 µl	0.5 mM of each dNTP
Upstream primer	Variable	0.3-0.4 µM
Downstream primer	Variable	0.3-0.4 µM
tiAmplus DNA Polymerase, 2.5 U/µl	0.6—0.8 µl	1.5—2 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradient when frozen.
2. Prepare reaction mixes at room temperature. Use of tiAmplus DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. For tiAmplus DNA Polymerase-based PCR, optimal concentration of $MgSO_4$ in PCR reaction is 2.9 mM (as provided with the 1 x Amplus Buffer) when using Eurx 0.5 mM dNTP (each) and recommended amount of DNA template (see point 7). In most cases this concentration will produce satisfactory results. If increased Mg^{2+} concentration is needed use 25 mM $MgSO_4$ provided and increase Mg^{2+} concentration in increments of 0.1 mM up to 3.5 mM.
5. 1.75 U of tiAmplus DNA Polymerase is recommended starting concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. Too much enzyme may generate artifacts like as smearing of bands, etc. In some cases it may be necessary to optimize the enzyme concentration in range 1.5–2 U to achieve its best performance (try 0.6, 0.7, 0.8 µl of the enzyme per 50 µl PCR reaction).
6. The recommended concentration of primers is 0.3-0.4 µM. This concentration enables to achieve satisfying yield while not compromising amplification specificity from genomic targets.
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 and 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 performing long PCR amplification.
11. In most cases there is no need to add additives to 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.

Thermal Cycling Conditions for Products 0.1-10 kb

Step	Temperature	Time	Number of Cycles
Initial Denaturation	93-94°C	2 min	1
Denaturation	93-94°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 over 10 kb

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 in each additional cycle	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

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 have a length of 22-34 and should have annealing temperatures above 60°C to enhance reaction specificity.
3. When amplifying long PCR products keep denaturation temperature as low as possible. **For genomic DNA over 15 kb denaturation temperature of 92°C is strongly recommended** (genomic DNA is much more liable to breaks than episomal DNA, especially at 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 elongation of extension step (+20 s in each additional cycle starting from 11th cycle) is optional and allows to achieve better yield. For PCR products over 10 kb the elongation of extension step is necessary to achieve satisfying results due to loss of processivity of the enzymes blend.

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