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Polymerase X

TI Hybrid DNA Polymerase

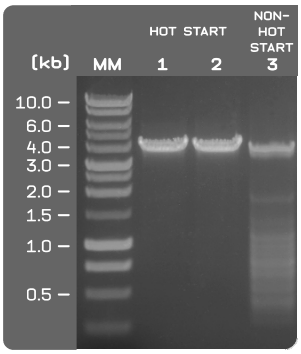
TI Hybrid DNA Polymerase

Extremely accurate and fast thermostable "Hot Start" DNA polymerase with superior template DNA binding specificity, enabling efficient high fidelity PCR of genomic targets up to 12 kb and episomal targets up to 20 kb.

Cat. No.	Size
E2940-01	100 units
E2940-02	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 Hybrid DNA Polymerase.
PCR amplification using EURx tiHybrid DNA Polymerase. A 4 kb amplicon of the human beta-globin gene was amplified using EURx tiHybrid DNA Polymerase, 10 x Hybrid Buffer and 0.2 mM dNTPs in 50 µl reaction volume.
Lane MM: molecular size marker – Perfect Plus 1 kb DNA Ladder (Cat. No. E3131).
Lanes 1,2: PCR amplification reactions using 1 U tiHybrid DNA Polymerase (Hot Start). Reactions were incubated 30 min at 25˚C before PCR.
Lane 3: PCR amplification reactions using 1 U Hybrid DNA Polymerase (non-Hot Start). The reaction was incubated 30 min at 25˚C before PCR.

Description:

- tiHybrid DNA Polymerase, a new generation hot start enzyme, is blocked at moderate temperatures and thus allows PCR reaction assembly at room temperature. Polymerase activity is restored during normal cycling conditions.
- Automated "Hot Start" using tiHybrid DNA Polymerase leads to an increase of PCR specificity, sensitivity and yield in comparison to conventional PCR assembly.
- tiHybrid is a genetically engineered thermostable DNA polymerase.
- Ultrapure recombinant enzyme.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5' 3' direction in the presence of magnesium ions.
- The enzyme exhibits 3´→5´ proofreading activity, resulting in more than 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to use shorter extension times.
- The modification of Hybrid DNA Polymerase enhances the target length capability of *Pfu*Plus! DNA Polymerase with regard to genomic targets (up to 12 kb from human genomic DNA).
- **Due to the genetic modification of the polymerase, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols.**
- tiHybrid DNA Polymerase is recommended for general use in PCR, use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures and cloning of blunt-ended PCR products.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22˚C), 100 mM KCl, 0.5 % Tween™20, 0.5 % Igepal CA-630, 0.1 mM EDTA, 1 mM dithiothreitol, 50 % glycerol and stabilizers.

10 x Reaction Buffer:

10 x Hybrid Buffer
The buffer contains 15 mM $MgCl_2$.

Quality Control:

All preparations are assayed for contaminating 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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TI Hybrid DNA Polymerase

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Hybrid Buffer, containing 15 mM $MgCl_2$.	5 µl	1x
dNTP mix (5mM each)	2.0 µl	0.2 mM each dNTP
Upstream primer	Variable	0.5 µM
Downstream primer	Variable	0.5 µM
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
DMSO (optional)	1-5 µl	2-10 % (v/v)
tiHybrid DNA Polymerase, 2 U/µl	0.5 µl	1 U
Total volume	50 µl	-

Thermal Cycling Conditions for Products up to 10 kb in Size:

Step	2-step protocol		3-step protocol		Number of Cycles
	Temperature	Time	Temperature	Time	
Initial Denaturation	98˚C	30 s	98˚C	30 s	1
Denaturation	98˚C	5-10 s	98˚C	5-10 s	25-35
Annealing	-	-	X˚C	10-30 s	
Extension					
a. general (genomic targets and episomal targets over 2 kb)	72˚C	30 s /1 kb	72˚C	30 s / 1 kb	
b. episomal targets up to 2 kb	72˚C	15-20 s / 1 kb	72˚C	15-20 s / 1 kb	
Final Extension	72˚C	5-7 min	72˚C	5-7 min	1
Cooling	4˚C	Indefinite	4˚C	Indefinite	1

Thermal Cycling Conditions for Products Larger Than 10 kb in Size:

Step	2-step protocol		3-step protocol		Number of Cycles
	Temperature	Time	Temperature	Time	
Initial Denaturation	92-93˚C	2 min	92-93˚C	2 min	1
Denaturation	92-93˚C	10 s	92-93˚C	10 s	25-35
Annealing	-		X˚C	10-30 s	
Extension	72˚C	30 s /1 kb	72˚C	30 s /1 kb	
Final Extension	72˚C	5-7 min	72˚C	5-7 min	1
Cooling	4˚C	Indefinite	4˚C	Indefinite	1

PCR Assembly - Notes:

1. **Concentration Differences.** Completely thaw and mix thoroughly all components of the PCR reaction prior to use for avoiding localized differences in salt concentration. This is especially important for magnesium solutions, because they form concentration gradients when frozen.
2. **Room Temperature.** Setup reactions at room temperature. Use of tiHybrid DNA Polymerase allows room temperature reaction setup. Mix well.
3. **Cycler Preheating Not Required.** Place assembled reactions in a thermal cycler that has been preheated to denaturation temperature.
4. **Add Enzyme as Last Component:** tiHybrid DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs, proofreading activity of tiHybrid DNA Polymerase may degrade primers.
5. **$MgCl_2$:** For tiHybrid DNA Polymerase-based PCR, standard concentration of $MgCl_2$ is 1.5 mM (as provided with 1 x Hybrid Buffer). In most cases this concentration will produce satisfactory results. For some PCR targets (especially cDNA targets) $MgCl_2$ optimization may be required. If increased Mg_2^+ concentration is needed, use the provided 25 mM $MgCl_2$ solution to adjust the concentration.
6. **Enzyme Amount.** 1 U of tiHybrid DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, the enzyme will be in excess and will produce satisfactory results. For some PCR targets further optimization will be required. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
7. **High quality dNTPs** should be used for optimal performance with Hybrid DNA Polymerase.
8. **Additives / PCR Enhancers.** In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures and long targets additives such as DMSO (provided) can be included to improve amplification. The recommended starting DMSO concentration (if needed) is 3 %.
9. **Template DNA Amount.** The amount of DNA template required depends on the type of DNA being amplified. Generally 50-250 ng of genomic DNA, 0.1-10 ng of plasmid DNA, 1-20 ng of phage DNA and 10-100 ng of multicopy chromosomal genes is recommended.

PCR Cycling - Notes:

1. **Initial Denaturation.** A 30-second initial denaturation at 98˚C is recommended for most targets up to 10 kb. The initial denaturation time can be extended up to 3 min in case of tempates that require longer denaturation. Denaturation at lower temperatures (92-93˚C) allows to achieve higher yield for long targets over 10 kb.
2. **On Primer Annealing Temperature.** tiHybrid DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (Tm) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. Tm's should be calculated with the base-stacking method (nearest-neighbor method). Use the calculator of the base-stacking method on the Roboklon website (http://www.roboklon.de/eurx/hybrid). Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg2+ concentration. As a basic rule, for primers >20nt, use an annealing temperature at a Tm +3˚C of the lower Tm primer. For primers 20nt, use an annealing temperature equal to the Tm of the lower Tm primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. **Performing Combined Annealing / Extension Steps.** A 2-step protocol allows to perform a combined annealing/extension step at 72˚C and is recommended for primers with Tm values of at least 69˚C (>20nt) or 72˚C (20nt). The 2 step protocol allows to save time of PCR reaction.
4. **Extension Time.** An extension time of 30 s/ 1 kb is recommended for most targets. In some cases (episomal targets up to 2 kb) a shorter extension time of 15-20 s/ 1 kb may not affect PCR yield and allow to save amplification time.

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