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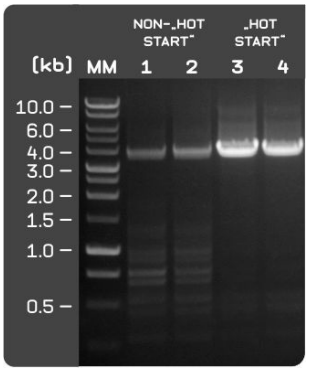
TI OptiTaq DNA Polymerase

Taq DNA Polymerase
Pyrococcus sp. DNA Polymerase
Polymerase Enhancing Factor

Cat. No.	Size
E2725-01	100 units
E2725-04	500 units
E2725-02	1000 units
E2725-03	5000 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 tiOptiTaq DNA Polymerase - demonstration of "HotStart" capabilities.
A 4 kb amplicon of the human alpha-globin gene was amplified using EURx tiOptiTaq DNA Polymerase, 10 x Pol Buffer B and 0.2 mM dNTPs in 50 µl reaction volume.
Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder (E3131).
Lane 1,2: PCR amplification reactions using 1.25 U non-"HotStart" OptiTaq DNA Polymerase (E2600). Reactions were incubated for 30 min at 25˚C before PCR.
Lane 3, 4: PCR amplification reactions using 1.25 U "HotStart" tiOptiTaq DNA Polymerase. Reactions were incubated for 30 min at 25˚C before PCR.

"Hot Start" mixture of thermostable DNA polymerases: *Taq* DNA Polymerase and proofreading *Pyrococcus* sp. DNA Polymerase. The blend generates amplicons up to 20 kb in length, with stringent amplification specificity as well as high sensitivity, fidelity and yield.

Description:

- tiOptiTaq DNA polymerase is a ew generation "HotStart" enzyme blend. Enzyme activity is blocked at moderate temperatures and allows room temperature reaction setup. DNA polymerase actvity is restored during normal PCR cycling conditions.
- tiOptiTaq DNA Polymerase is a modified, optimized and balanced thermostable enzymes blend containing top quality *Taq* DNA polymerase, *Pyrococcus* sp. DNA polymerase and a polymerase-enhancing factor.
- Ultrapure, recombinant enzymes are used to prepare tiOptiTaq DNA Polymerase.
- Use of tiOptiTaq DNA Polymerase allows for an enormous increase of PCR specificity, sensitivity and yield, as compared to conventional PCR assembly methods.
- The blend catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium ions and exhibits 3'→5' proofreading activity, resulting in considerably higher PCR fidelity and processivity in comparison to non-modified *Taq* DNA polymerase.
- Enables increased amplification product yield over a wide range of PCR products, as compared to *Taq* DNA polymerase.
- Improves PCR results with critical templates, such as containing GC-rich regions.
- Maintains 5' → 3' exonuclease activity.
- Adds extra A to a fraction of 3' ends, thus enabling both, TA- and blunt end cloning, respectively.
- tiOptiTaq DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products up to 20 kb in length.

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 and stabilizers.

10 x Reaction Buffer:

10 x Pol Buffer A (optimalization buffer without $MgCl_2$):
The buffer allows to optimize $MgCl_2$ concentration.

10 x Pol Buffer B (general application, up to 20 kb):
The buffer contains 15 mM $MgCl_2$ and is optimized for use with 0.2 mM of each dNTP.

10 x Pol Buffer C (coloured):
Similar to 10 x buffer B, but additionally enriched with two gel tracking dyes and a gel loading reagent. The buffer enables direct loading of PCR products to agarose gels.

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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PCR PROTOCOL (1)

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Pol Buffer A or 10 x Pol Buffer B or 10 x Pol Buffer C	5 µl	1x
25 mM MgCl2	2 - 10 µl when using 10 x Pol Buffer A or 0 - 7 µl when using 10 x Pol Buffer B or 10 x Pol Buffer C	1 - 5 mM 1.5 - 5 mM
dNTP mix (5mM each)	2 µl	0.2 mM each dNTP
Upstream primer	Variable	0.3 - 0.5 µM
Downstream primer	Variable	0.3 - 0.5 µM
tiOptiTaq DNA Polymerase, 2.5 U/µl	0.5 µl	1.25 U
Template DNA	Variable	‹ 0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Notes:

1. **Concentration Differences.** 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. **Room Temperature.** Setup reactions at room temperature. Use of tiOptiTaq DNA Polymerase allows room temperature reaction setup. Mix well.
3. **Cycler Preheating Not Required.** Reactions can be placed in a room temperature thermal cycler.
4. **$MgCl_2$.** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x Pol Buffer B or 1 x Pol Buffer C) when using 0.2 mM dNTP (each). In most cases these concentrations will produce satisfactory results. However, in some cases, reaction may be improved by determining optimal concentration of $MgCl_2$. Should the reaction require inceased Mg^{2+} concentrations, use the supplied 25 mM $MgCl_2$ solution for adjustment. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 50 µl will add 25 nmol $MgCl_2$ and thus increase total Mg^{2+} reaction concentration in 0.5 mM.
5. **Colored PCR Buffer.** 10 x Pol Buffer C allows PCR reactions to be loaded directly onto an agarose gel without prior addition of gel loading buffer. The buffer is identical to 10 x Pol Buffer B, but contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye), which separate during electrophoresis. In a 1% [w/v] agarose gel, the red dye migrates at the same rate as 600 bp DNA fragments and the yellow dye migrates faster than 20 bp. Both dyes do not interfere with most downstream enzymatic applications, however, it is recommended to purify PCR products prior to subsequent enzymatic treatment.
6. **Enzyme Amount.** 1.25 U of tiOptiTaq DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. In some cases it may be necessary to optimize the enzyme concentration.
7. **Template DNA Amount.** As a general guide for how much template DNA to use, start with a minimum of 10^4 target sequence copies to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.1 x 10^{11} molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10^8 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules).
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 and targets with complex secondary structures, additives such as DMSO may be included to improve amplification. Use DMSO in a concentration range between 2-8% [v/v]. The recommended starting DMSO concentration (if required) is 3% [v/v].
9. **Long Range PCR – Template DNA Amount.** For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
10. **Template DNA Quality.** 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.
11. **Template DNA Storage.** 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.
12. **Thin Walled PCR Tubes.** Use only thin-walled 0.2 ml tubes when performing long PCR amplification.

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PCR PROTOCOL (2)

Thermal Cycling Conditions for Products between 0.1 and 10 kb in Size:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	93-95˚C	2-5 min	1
Denaturation	93-95˚C	15-30 s	25-35
Annealing	50-68˚C	30 s	
Extension	72˚C or 68˚C	1 min/1 kb	
Final Extension	72˚C or 68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Thermal Cycling Conditions for Products Exceeding 10 kb in Size:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	92-94˚C	2 min	1
Denaturation	92-94˚C	10-15 s	10
Annealing	60-68˚C	30 s	
Extension	68˚C	1 min/1 kb	
Denaturation	92-94˚C	10_15 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. **Initial Denaturation:** A 2 min initial denaturation step at 93-95˚C is required to release the thermal dependent inhibitor and to restore DNA polymerase activity.
2. **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.
3. **Primer Length.** Typical primers for long PCR amplification reactions have a length of 22-34 nt and should have annealing temperatures above 60˚C to enhance reaction specificity.
4. **Long PCR – Short Denaturation Steps.** When amplifying long PCR products, keep denaturation steps as short as possible and denaturation temperature as low as possible (try not to exceed 2 min at 94˚C during initial denaturation and 10-15 s at 94˚C during cycle denaturation step). Sometimes fragments with high GC-content need higher denaturation temperatures, but keep in mind that the yield increases when denaturation temperature / duration is decreased.
5. **Long PCR – Low Elongation Temperature.** For PCR products exceeding 5 kb in length an elongation temperature of 68˚C is strongly recommended**.**
6. **Long PCR – Extended Elongation Time.**For PCR products exceeding 10 kb in length, an elongation of the extension step (+20 s in each additional cycle, starting from the 11th cycle) is strongly recommended to compensate for loss of processivity of the enzymes blend.

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