EURx® MOLECULAR BIOLOGY PRODUCTS

tiTaq DNA Polymerase HOT START

Cat. No.	size
E2715-01	200 units
E2715-04	500 units
E2715-02	1000 units
E2715-03	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 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 tiTaq DNA Polymerase. A 6.9 kb amplicon of Bacillus phage DNA was amplified with tiTaq DNA Polymerase. Reactions were incubated at 25°C for 30 min before amplification.
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder.
Lanes 1, 2: PCR amplification reactions using 1.25 U tiTaq DNA Polymerase, Pol Buffer B and 0.2 mM dNTPs in 50 µl reaction volume.
Lane 3: PCR amplification reaction using 1.25 U Taq DNA Polymerase, Pol Buffer B and 0.2 mM dNTPs in 50 µl reaction volume.

Description:

- tiTaq DNA Polymerase is a new generation "hot start" enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during normal cycling conditions.
- Use of tiTaq DNA Polymerase allows for the increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- Automatic "hot start" PCR is a fast and convenient method when assembling multiple PCR reactions.
- Both increased specificity and reduced mispriming improve multiplex PCR.
- Eliminated risk of template cross-contamination and assured safe laboratory practice, due to removed necessity to open hot tubes.
- Thermostable tiTaq DNA Polymerase replicates DNA at 72°C and exhibits a half-life of 40 min at 95°C.
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.
- tiTaq 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 10 kb.

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 Buffers:

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

10 x Pol Buffer B (general application, up to 10 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 (colored):

10 x Pol Buffer B enriched with two gel tracking dyes and a gel loading reagent.

The buffer enables direct loading of PCR products onto an agarose gel.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and non-specific 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 Pol Buffer A or 10 x Pol Buffer B or 10 x Pol Buffer C	5 µl	1x
25 mM $MgCl_2$	2-10 µl when using 10 x Pol Buffer A	1-5 mM
	0-7 µl when using 10 x Pol Buffer B or 10 x Pol Buffer C	1.5-5 mM
dNTP mix (5 mM each)	2 µl	0.2 mM of each dNTP
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 µM
tiTaq 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. 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 tiTaq DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided by the 1 x Pol Buffer B or the 1 x Pol Buffer C) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, in some cases, reaction may be improved by determining optimal concentration of $MgCl_2$.
5. The 10 x Pol Buffer C allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The buffer contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
6. 1.25 U of tiTaq DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
7. 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 additives such as DMSO can be included to improve amplification. Use DMSO in concentrations of 2-8%. The recommended starting DMSO concentration (if needed) is 3%.
8. As a general guide for how much template DNA to use, start with a minimum 10^4 copies of the target sequence 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).

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation	94-95°C	15-30 s	25-35
Annealing	50-68°C	30 s	
Extension	72°C	1 min/1 kb	
Final Extension	72°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. When amplifying long PCR products (over 5 kb):
   a. initial denaturation should be 2 min at 94°C
   b. cycle denaturation should be 15-20 s at 94°C
   c. use an elongation temperature of 68°C instead of 72°C.

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