TIARIS™ Pfu DNA Polymerase Kit

Ordering Info

TBK0085, 12.5 U (2.5U/ µL)(sample)

TBK0086, 125 U (2.5U/ µL)

TBK0087, 500 U (2.5U/ µL)

Description

TIARIS™ Pfu DNA Polymerase Kit is an ideal kit when high fidelity is an important requirement in your PCR. It includes the proofreading enzyme TIARIS™ Pfu DNA polymerase and the highly purity High-Q™ dNTPs. TIARIS™ Pfu DNA polymerase (90 kDa) is a high fidelity polymerase from *Pyrococcus furiosus* produced in *Escherichia coli*. The enzyme has a strong 5'→ 3' polymerase activity and 3'→5' exonuclease (proofreading) activity which corrects nucleotide incorporation errors, thereby increasing fidelity and accuracy of DNA polymerization.

Features

- **High accuracy and fidelity PCR** with an error rate of $1.3x10^{-6}$ errors/bp per cycle[1].
- **Elongation velocity is 0.2~0.4 kb/min (70~75°C).**
- **Suitable for blunt cloning purposes**, the enzyme produces blunt-end PCR fragments.
- **Highly thermostable enzyme,** which retains 94-99% of its activity after 1 hour at 95°C.
- **PCR amplification > 2 kb require optimization.**

Applications

- Broad range of techniques that require high fidelity PCR products such as: cloning, expression of gene of interest, mutagenesis.
- Generation of PCR fragments for blunt cloning.
- Filling 5´protruding ends.

Kit Components

Components	TBK0086	TBK0087
TIARIS™ Pfu DNA polymerase (2.5 U/ µL)	50 µL	200 µL
Pfu PCR Buffer (10x)	1.5 mL	1.5 mL
MgCl2 25 mM	1.5 mL	1.5 mL
High-Q™ dNTPs 10mM TOTAL	0.2mL	1 mL

Order Info Kit Components: *TIARIS™ Pfu DNA polymerase (TBZ0212) | Pfu PCR Buffer 10x (TBB0314) | MgCl2 25 mM (TBR0215) | High-Q™ dNTPs 10 mM TOTAL (TBR0209).*

Storage

Store at -20°C. Shipped in blue ice.

Unit Definition

One unit of TIARIS™ Pfu DNA polymerase catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 72°C.

Quality Control

Functionally tested in a 0.5kb PCR amplification (GC 52%).

Material required (not supplied)

- PCR Grade Water (TBB0303)
- PCR Tubes
- Primers

[1] *Cline J, Braman JC, Hogref HH (1996) Nucleic Acids Res 24 (8): 3546–3551.*

PROTOCOL

1. Thawing all components on ice. Vortex them and centrifugate.

2. On ice, prepare a mix of the following components, considering the number of samples plus two extra reactions.

Reaction Components	Final Concentration	Volume	Volume
TIARIS™ Pfu PCR Buffer 10x	1 x	2 µL	5 µL
MgCl2 25 mM	2.5 mM	2 µL	5 µL
dNTPs Mix 10 mM TOTAL	0.8 mM	1.6 µL	4 µL
Forward Primer (15 pmol/ µL)	0.2-0.75 µM	0.3-1 µL	1.7-2.5 µL
Reverse Primer (15 pmol/ µL)	0.2-0.75 µM	0.3-1 µL	1.7-2.5 µL
TIARIS™ Pfu DNA polymerase (2.5 U/µL)	0.05 U/ µL	0.4 µL	1 µL
Water, molecular biology grade		up 20 µL*	up 50 µL*
DNA template (add in step 4)		*	*
Final Volume		**20 µL**	**50 µL**

** consider volume of template to be added in step 4.*

3. Distribute the mix prepared in each PCR tube or well.

4. Add in each tube the DNA sample (plasmid DNA, cDNA: 10-75 ng; gDNA: 100-500 ng). Mix well.

5. Set up thermocycler with the following suggested parameters,

Process	Cycles	Temperature	Time
Denaturation	25 - 30 x	94-98 °C*	0:40
Annealing		Tm - 5°C	0:40
Extension		72 °C	2:00 per kb
Final Extension	1 x	72 °C	10:00
Conservation	1 x	4 °C	∞

** Use 98°C, if GC content is high.*

6. Store the PCR samples at -20°C.