TIARIS™ High-Fidelity DNA Polymerase kit

Ordering Info

TBK1030, 250 U (2U/ µL)

Description

TIARIS™ High-Fidelity DNA polymerase is a robust enzyme with 3´→ 5´ exonuclease (proofreading) activity and enhanced DNA binding, resulting in improved processivity, yield, and extremely low error-rate. TIARIS™ High-Fidelity DNA polymerase has an error-rate of approximately 1 error per $4.4x10^7$ nucleotides incorporated, which is 50x lower than Taq DNA polymerase. The enzyme is ideal for applications such as high-fidelity PCR, site-directed mutagenesis, crude sample PCR, blunt-end cloning, among others, where robustness and proofreading are important. The supplied reaction buffer (5x) includes not only 15 mM $MgCl_2$, but also 5mM dNTPs, enhancers, and stabilizers, optimized to increased PCR success rates.

Features

- High fidelity (50X Taq)
- Fast PCR due to short extension rate: 2kb/min.
- Suitable for blunt cloning purposes, the enzyme produces blunt-end PCR fragments.
- Robust performance, minimal optimization needed

Applications

- High-Fidelity PCR, blunt-end cloning, site-directed mutagenesis.

Kit Components

Components	TBK0086
TIARIS™ High-Fidelity DNA Polymerase (2 U/ µL)	125 µL
PCR Buffer (5x) [containing $MgCl_2$ and dNTPs]	3 x 1 mL

Storage

Store at -20°C in a constant temperature freezer.

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

Quality Control

Functionally tested in PCR. Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays.

PROTOCOL

1. Thawing all components on ice. Gently vortex and briefly centrifuge kit components after thawing.

2. On ice, prepare a mix of the following components for each 25 µL reaction, considering the number of samples plus two extra reactions:

Reaction Components	Final Concentration	Volume
PCR Buffer 5x*	1 x	5 µL
Forward Primer (5 pmol/ µL)	0.4 µM	2 µL
Reverse Primer (5 pmol/ µL)	0.4µM	2 µL
TIARIS™ High-Fidelity DNA polymerase (2 U/µL)	0.02-0.04 U/µl	0.25-0.50 µL
Water, molecular biology grade		up 25 µL**
DNA template (add in step 4)		**
Final Volume		**25 µL**

** The PCR Buffer already includes dNTPs and has already been optimized with respect to the MgCl2 concentration and other components to maximize success rates. It is not recommended to add additional MgCl2 or other PCR enhancers.*

*** 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 (In case of cDNA <100ng and in case of gDNA <500ng). Mix well.

5. Set up thermocycler with the following suggested parameters,

Process	Cycles	Temperature	Time
Initial Denaturation	1 x	95 °C	01:00
Denaturation	25 - 35 x	95 °C	0:15
Annealing		Ta*	0:15
Extension		72 °C	0:30 per kb
Final Extension	1 x	72 °C	03:00
Hold	1 x	4 °C	∞

** Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm.*

6. Store the PCR samples at -20°C.