TIARIS™ High-Fidelity DNA Polymerase kit

Ordering Info

TBK1034, 100 U (2U/ µL)

Description

TIARIS™ High-Fidelity DNA Polymerase is a robust enzyme with 3′→5′ exonuclease (proofreading) activity and enhanced DNA-binding properties, resulting in improved processivity, higher yields, and an extremely low error rate. Protein engineering has enhanced its DNA-binding affinity, enabling faster DNA extension rates (2–6 kb/min) and rapid PCR amplification.

TIARIS™ High-Fidelity DNA polymerase exhibits a 100-fold lower error rate 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 (100x Taq DNA polymerase)
- Fast PCR due to short extension rate: 2-6kb/min.
- Amplicon size: up to 20 kb.
- 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	TBK1034
TIARIS™ High-Fidelity DNA Polymerase (2 U/ µL)	50 µL
PCR Buffer (5x) [containing $MgCl_2$ and dNTPs]	1.7 mL
Tiaris™ PCR Enhancer	1.7 mL

Order Info Kit Components: *TIARIS™ High-Fidelity DNA Polymerase (TBZ1034) | PCR Buffer (5x) (TBB1034).*

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.

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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
Tiaris™ PCR Enhancer** *(Optional)*	1 x	2.5 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 $MgCl_2$ concentration and other components to maximize success rates. It is not recommended to add additional $MgCl_2$ or other PCR enhancers.*

** *in case of difficult and/or long target (e.g., GC rich or with complex secondary structures)*

*** *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.

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