WARM™ Fast Hot-Start DNA Polymerase Kit

Ordering Info

TBK1026, 250 U

TBK1027, 500 U

Description

WARM™ Fast Hot-Start DNA Polymerase Kit is a convenient kit to speed amplification of DNA samples. The kit includes the robust WARM™ Fast Hot-Start polymerase, characterized by its extension rate of 2 sec/ kb and an optimized buffer composed of $MgCl_2$ 15 mM, highly purity High-Q™ dNTPs 5 mM each, and enhancers. WARM™ Hot-Start DNA polymerase is a modified polymerase suitable for amplification from unpurified samples or difficult targets.

Kit Components

Components	TBK1026	TBK1027
WARM™ Fast Hot-Start DNA Polymerase (5 U/µL)	50 µL	2 x 50µL
5x WARM™ Fast Hot-Start Buffer	2 x 1 mL	4 x 1 mL
PCR Grade Water, nuclease free	2 mL	2 x 2 mL

Order Info Kit Components: *WARM™ Fast Hot-Start DNA Polymerase (TBK1026-1) | 5x WARM™ Fast Hot-Start Buffer (TBK1026-2) | PCR Grade Water, nuclease free (TBB0303).*

Features

- Amplification up 5 kb amplicons.
- Extension rate 2 seconds per kb (< 1kb) and 15 seconds per kb (> 1 kb to 5 kb).
- Suitable for TA cloning purposes, the enzyme adds 3´adenine at the end of PCR fragment.
- Activation Controlled, inactive at low temperatures and fully activated at high temperature.

Storage

Store at -20°C protected from light. Shipped in blue ice.

Quality Control

Functionally tested in a PCR amplification.

Applications

- Amplification of complex targets.
- Direct PCR of unpurified samples (*blood, urine*).
- Generation of PCR fragments for TA cloning.
- Multiplex PCR.

Material required (*not supplied*)

- PCR Tubes
- Primers

PROTOCOL

I. PREPARING REACTIONS

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a tube on ice and add the following components for each 25 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
5x WARM™ Fast Hot-Start Buffer	5 µL	1x
Forward primer 5 µM (5 pmol/µL)	2µL	0.4 µM
Reverse primer 5 µM (5 pmol/µL)	2 µL	0.4 µM
PCR Grade Water, nuclease-free	up 25 µL	
Template DNA		(1)
WARM™ Fast Hot-Start DNA Polymerase (5 U/µL)	0.1 µL	0.5 U (2)
Final Volume	25 µL	

(1) *Optimal amounts of template: genomic DNA ≤250 ng, cDNA synthesis reaction 1-5 µL (≤50 ng), direct PCR of unpurified samples from urine or blood 1 µL.*
(2) *Higher enzyme concentration may be required for difficult amplifications (up 2.5 U)*

3. Aliquot the master mix into individual PCR tubes or in well-plate.
4. Gently vortex and spin down the samples. Add template DNA.

II. PCR SETUP

1. Perform PCR using standard thermal cycling conditions:

Step	Cycles	Temperature	Time
Activation & Initial Denaturation	1 x	95 °C	3:00
Denaturation	40 x	95 °C	0:15
Annealing		Tm	0:15
Extension		72 °C	sec per kb*
Final Extension	1 x	72 °C	3:00
Conservation	1 x	4 °C	∞

* *< 1kb, 2 seconds per kb; >1-5 kb, 15 seconds per kb; multiplex PCR, 90 seconds per kb independent of size.*

2. Store the samples at -20°C until use.