WARM™ FAST Hot-Start Master Mix (2x)

Ordering Info

TBK1028, 80 reactions (1 mL)

TBK1029, 400 reactions (5 x 1 mL)

Description

WARM™ FAST Hot-Start Master Mix (2x) is a ready-to-use master mix containing an engineered DNA polymerase that combines hot-start capability, high processivity, and ultra-fast extension speed (2 sec/kb). The buffer composition has been optimized through high-throughput screening, resulting in enhanced resistance to common PCR inhibitors. PCR product is fully compatible with downstream applications.

Features

- Ready to use.
- Fast Amplification of PCR targets.
- High Extension Rate, 2 seconds/ kb for targets < 1 kb.
- Generation of PCR fragments for TA cloning.
- Built-In Hot Start.

Applications

- Fast PCR.
- Hot-start PCR.
- Genotyping.
- Multiplex PCR. *Fast cycling conditions should not be applied in multiplex PCR.*
- Direct PCR from crude samples such as blood, urine, and bacterial colonies.
- Fast amplification of complex targets (including GC-rich and AT-rich templates).

Kit Components

Components	TBK1028	TBK1029
WARM™ FAST Hot-Start Master Mix(2x)	1 mL	5 x 1 mL
PCR Grade Water	2 mL	3 x 2 mL

Order Info Kit Components: *WARM™ FAST Hot-Start Master Mix (TBK1028) | PCR Grade Water (TBB0303).*

Storage

Store at -20°C. Shipped in blue ice.

Quality Control

Functionally tested in a 1 kb fast PCR amplification protocol. The resulting PCR product is visualized as single band in agarose gel.

Material required (*not supplied*)

- PCR Tubes
- Specific Primers

Also available:

- STOUT™ FAST DNA Polymerase (TBK1011, TBK1012)
- STOUT™ Recombinant Taq DNA Polymerase Master Mix (2x) (TBK0028, TBK0029)

PROTOCOL

This protocol serves as a guideline for PCR amplification. Optimal cycling conditions such as incubation times, temperatures, and amount of template DNA depend on the DNA target (GC-content, size, quantity, purity, etc), primers, and must be determined individually. We recommend starting with the basic protocol described below and subsequently optimize the annealing temperature, incubation times and cycling numbers.

1. Gently mix and briefly centrifuge the master mix after thawing.

2. Prepare a PCR master mix containing the appropriate volume of all reaction components, considering the number of samples plus two extra reactions. Calculate the required volume of each component based on the following table:

Reaction Components	Final Concentration	Volume
WARM™ FAST Hot-Start Master Mix (2x)	1 x	12.5 µL
Forward Primer (10 pmol/ µL)	0.4 µM	1 µL
Reverse Primer (10 pmol/ µL)	0.4 µM	1 µL
PCR Grade Water		up 25 µL*
DNA template (add in step 4)		*
Final Volume		**25 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 (cDNA: < 50 ng; gDNA: < 250 ng). Mix well.

5. Set up thermocycler:

Process	Cycles	Temperature	Time
Initial denaturation	1 x	95 °C	3 min
Denaturation	40 x	95 °C	15 sec
Annealing		Ta*	15 sec
Extension		72 °C	2 sec per kb (< 1 kb) 15 sec per kb (> 1 kb) **
Final Extension	1 x	72 °C	3 min
Conservation	1 x	4 °C	∞

***** Ta should be 2°C below the Tm value of the primer with the lowest Tm.

****** 90 seconds total per cycle in case of multiplex PCR, independent of the sizes of the amplicons.