WARM™ HotStart PCR Master Mix (2x)

Ordering Info

TBK0041, 5 reactions (sample)

TBK0042, 2 x 1.25 mL

TBK0043, 4 x 1.25 mL

Description

WARM™ HotStart PCR Master Mix is an optimized formulation to enable successful amplification in a process with reduced manipulation. The formula includes the hot-start enzyme WARM™ START Taq DNA polymerase and highly purity dNTPs. WARM™ HotStart Taq DNA polymerase is a thermostable polymerase from *Thermus aquaticus* produced in *Escherichia coli* reversibly inactivated by antibody binding. Once the enzyme is activated it has a strong 5´→3´ polymerase activity.

Features

- Ready to use, avoiding mistakes in PCR reaction preparation.
- Enhanced specificity and sensibility, amplifies low copy number targets with reduced non-specific
- Thermostable half-life at 94 °C is 40 minutes.
- 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 temperature > 70°C.
- Error Rate 1–20x10^{-5} errors/bp per cycle.

Applications

- Real time PCR to quantify DNA or cDNA targets, gene expression, SNPs.
- Genotyping
- Generation of PCR fragments for TA cloning.

Kit Components

Components	TBK0042	TBK0043
WARM™ HotStart PCR Master Mix (2x)	2 x 1.25 mL	4 x 1.25 mL
PCR Grade Water, nuclease free	2 x 2 mL	4 x 2 mL

Order Info Kit Components: *PCR Grade Water, nuclease free (TBB0330).*

Storage

Store at -20°C. Shipped in blue ice.

Unit Definition

One unit of WARM™ HotStart Taq DNA polymerase catalyzes the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74 °C.

Quality Control

- Functionally tested in a 1kb PCR amplification (GC 52%).

Material required (not supplied)

- PCR Grade Water (TBB0303)
- 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 50 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
WARM™ HotStart PCR Master Mix (2x)	25 µL	1x
Forward primer 15 µM (15 pmol/µL)	1 µL	0.75 µM
Reverse primer 15 µM (15 pmol/µL)	1 µL	0.75 µM
PCR Grade Water, nuclease-free	up 50 µL	
Template DNA		**
Final Volume	**50 µL**	

** *Optimal amounts of template: genomic DNA ≤500 ng, plasmid or viral DNA 1-50 ng, cDNA synthesis reaction 1-5 µL.*

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
Initial denaturation	1 x	94 °C	15:00
Denaturation	30 -40 x	94 °C	0:35
Annealing		Tm	0:35
Extension		72 °C	1:00 per kb
Final Extension	1 x	72 °C	10:00
Conservation	1 x	4 °C	∞

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