

## WARM™ HotStart Taq DNA Polymerase Kit

### Ordering Info

TBK0035, 20 U (5U/μL)(sample)

TBK0036, 500 U (5U/μL)

TBK0037, 1.000 U (5U/μL)

### Description

WARM™ HotStart Taq DNA Polymerase Kit is a convenient kit that 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

- **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<sup>-5</sup> 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.
- Amplification of PCR fragments up 5 kb.

### Kit Components

Components	TBK0036	TBK0037
WARM™ HotStart Taq DNA polymerase (5 U/μL)	100 μL	200 μL
WARM™ PCR Buffer (10x)	1.5 mL	2 x 1.5 mL
MgCl <sub>2</sub> 25 mM	1.5 mL	2 x 1.5 mL
High-Q™ dNTPs 10mM TOTAL	1 mL	2 x 1 mL

**Order Info Kit Components:** WARM™ PCR Buffer 10x (TBB0311) | MgCl<sub>2</sub> 25 mM (TBR0215) | High-Q™ dNTPs 10 mM TOTAL (TBR0209).

### 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

1. Thawing all components on ice. Vortex them and centrifugate.
2. On ice, prepare a mix of the following components, considering the number of samples plus two extra reactions.

Reaction Components	Final Concentration	Volume	Volume
WARM™ PCR Buffer 10x	1 x	2 µL	5 µL
MgCl <sub>2</sub> 25 mM	2.5 mM	2 µL	5 µL
dNTPs Mix 10 mM TOTAL	0.8 mM	1.6 µL	4 µL
Forward Primer (15 pmol/ µL)	0.2-0.75 µM	0.3-1 µL	1.7-2.5 µL
Reverse Primer (15 pmol/ µL)	0.2-0.75 µM	0.3-1 µL	1.7-2.5 µL
WARM™ HotStart Taq DNA polymerase (5 U/µL)	0.05 U/ µL	0.2 µL	0.5 µL
Water, molecular biology grade		up 20 µL*	up 50 µL*
DNA template (add in step 4)		*	*
<b>Final Volume</b>		<b>20 µL</b>	<b>50 µL</b>

\* 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 (plasmid DNA, cDNA: 10-75 ng; gDNA: 100-500 ng). Mix well.
5. Set up thermocycler,

Process	Cycles	Temperature	Time
Enzyme Activation	1 x	95 °C	05:00
Denaturation		94 °C	0:35
Annealing	30-40 x	T <sub>m</sub>	0:35
Extension		72 °C	1:00 per kb
Final Extension	1 x	72 °C	10:00
Conservation	1 x	4 °C	∞

6. Store the PCR samples at -20°C.