

## TECHNICAL SHEET:

# Kit PCR HOT START

Ref : PW11

**Concentration** : 5 units/ $\mu$ L

**Storage**: at  $-20^{\circ}\text{C}$  for two years

**Quantity** : 250 Units

### Description

This kit includes Hot Start Taq DNA Polymerase for increased specificity and reduced non-specific amplification, a 10X Hot Start Taq Buffer optimized for efficient enzymatic activity, high-purity 2.5 mM dNTPs for reliable DNA synthesis, and 6X DNA Loading Buffer for convenient sample preparation and gel analysis. Designed to deliver superior performance across a variety of PCR applications, the kit ensures robust amplification and consistent results. Store all components at  $-20^{\circ}\text{C}$  to maintain optimal performance.

### Highlights

- **Taq** Polymerase *HOT START* offers 18-fold fidelity as compared to *Taq* Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into pEASY-T vectors.
- Reduced nonspecific amplification and primer dimer formation.
- Different from Taq antibody, no risk of contamination from mammalian DNA.
- Different from chemical modification, long denaturing step is not needed.
- Amplification of genomic DNA fragment up to 15 kb.

### Applications

- Complex templates
- GC/AT-rich templates
- Multiplex PCR
- High yield PCR

### Unit Definition

One unit of **Taq** Polymerase *HOT START* incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at  $74^{\circ}\text{C}$ .

### Quality Control

**Taq** Polymerase *HOT START* has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of **Taq** Polymerase *HOT START* has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

### Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 50% glycerol, stabilizers

## Kit Contents

Component	Volume
<b>Taq</b> Polymerase <i>HOT START</i>	250 U
10× <b>Taq</b> <i>HOT START</i> Buffer	0.5 ml
2.5 mM High Pure dNTPs	0.5 ml
6x DNA Loading Buffer	0.5 ml

## Reaction Components

Component	Volume	Final Concentration
Template DNA	Variable	As required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
10× <b>Taq</b> <i>HOT START</i> Buffer	5 µl	1x
2.5 mM High Pure dNTPs	4 µl	0.2 mM
<b>Taq</b> Polymerase <i>HOT START</i>	0.5-1 µl	1.25x2.5 units
<b>Nuclease-free Water</b>	Variable	-
<b>Total volume</b>	50 µl	-

## Thermal cycling conditions

94°C	2-5min	} 30-35 cycles
94°C	30 sec	
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

## Notes

- A final concentration of 2 mM MgSO<sub>4</sub> is sufficient for most targets amplification. For some targets, more Mg<sup>2+</sup> may be required.
- For optimal results, we recommend to use the 100 mM MgSO<sub>4</sub> stock to prepare a titration from 2 mM to 4 mM (final concentration) in 0.25 mM increments.
- 0.5 µl (2.5 units) enzyme is enough for per 50 µl reaction. For better amplification, up to 1 µl (5 units) enzyme can be used.
- For amplification of GC/AT-rich templates and complex templates, we suggest to use GC Enhancer.