

# QKGEN® HotStart DNA Polymerase

## Product description

QKGEN® HotStart DNA Polymerase is a novel hot-start enzyme. It uses two proprietary DNA binding proteins that at room temperature bind to the double-strand DNA template and primer, effectively neutralizing the DNA polymerase activity until denaturation occurs. As the denaturation step proceeds, the two proteins are inactivated, and the released primers and templates participate in the amplification reaction, enhancing PCR amplification efficiency.

### Highlights

- Fidelity is 18 times that of QKGEN® Taq DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Specificity is superior to antibody blocking and chemical blocking of hot-start DNA polymerases.
- Prepare reactions at room temperature to reduce non-specific amplification and primer dimers.
- The use of Taq antibodies reduces the risk of potential DNA contamination from mammals.
- Unlike chemically modified Taq, no heating steps are required.
- Amplification of genomic DNA fragments( $\leq 15$ kb).

### Unit Definition

One unit of QKGEN® HotStart DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

### Quality Control

- Functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE.
- Each batch of the HotStart Taq DNA Polymerase has been assayed for amplification efficiency of the p53 gene from 10ng 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

## Specifications

Name	Cat. No.	QAP141-1	QAP141-2	QAP141-3
QKGEN® HotStart DNA Polymerase	Size	500U	1000U	3000U

## Components

Name	QAP141-1	QAP141-2	QAP141-3
HotStart DNA Polymerase (2.5 U/ $\mu$ L)	500U $\times$ 1	500U $\times$ 2	500U $\times$ 6
10 $\times$ HotStart Buffer	1.2 mL $\times$ 2	1.2 mL $\times$ 4	1.2 mL $\times$ 12
2.5 mM dNTPs	800 $\mu$ L $\times$ 2	800 $\mu$ L $\times$ 4	1.2 mL $\times$ 8
10 $\times$ GC Enhancer	400 $\mu$ L	800 $\mu$ L	1.0 mL
6 $\times$ DNA Loading Buffer	1 mL	1 mL	1 mL $\times$ 2

[Note]

1. 10 $\times$ HotStart Buffer with 20 mM MgSO<sub>4</sub>: 200 mM Tris-HCl(pH 8.3), 200 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM MgSO<sub>4</sub>, others
2. GC Enhancer: For better amplification of GC/AT-rich or complex templates, we recommend adding GC enhancer into PCR reaction. GC enhancer is provided at 10 $\times$ concentration and can be used at 0.5 $\times$  - 5 $\times$ concentration.

## Storage

This product should be stored at -25~-15°C for 2 years.

## Application

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

## Recommended PCR reaction system

### 1.PCR reaction system

Components	Volume( $\mu$ L)	Final concentration
10 $\times$ HotStart Buffer	5	1 $\times$
HotStart DNA Polymerase(2.5 U/ $\mu$ L)	0.5-1	1.25-2.5U
Forward Primer(10 $\mu$ mol/L)	1	0.2 $\mu$ mol/L
Reverse Primer(10 $\mu$ mol/L)	1	0.2 $\mu$ mol/L
2.5mM dNTPs	4	0.2 mM
DNA	X	-
ddH <sub>2</sub> O	up to 50	

[Note]:

- 1) A final concentration of 2mM 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 100mM MgSO<sub>4</sub> stock to prepare a titration from 2 mM to 4mM(final concentration) in 0.25mM increments.
- 2) 0.5 $\mu$ L(1.25 units) enzyme is enough for per 50  $\mu$ L reaction. For better amplification, up to 1 $\mu$ L(2.5units) enzyme can be used.
- 3) For amplification of GC/AT-rich templates and complex templates, we suggest to use GC Enhancer.
- 4) If there is a little precipitation after the 10 $\times$ HotStart Buffer is thawed, please dissolve it in a 37 $^{\circ}$ C water bath and mix it for use.

### 2.Reaction program

Cycle step	Temp.	Time	Cycles
Pre-denaturation	94 $^{\circ}$ C	2-5 min	1
Transgender	94 $^{\circ}$ C	30 sec	30-35
Annealing	50-60 $^{\circ}$ C	30 sec	
Extension	72 $^{\circ}$ C	1-2 kb/min	
Extended reach	72 $^{\circ}$ C	5-10 min	1

## Notes

1. For your safety and health, please wear a lab coat and disposable gloves when operating.
2. This product is for scientific research purposes only!