QKGEN® High-Fidelity DNA Polymerase

Product description

QKGEN® High-Fidelity DNA Polymerase based on Pyrococcus Furiosis DNA Polymerase, genetically engineered. This enzyme possesses 5'→3' DNA polymerase activity and 3'→5' exonuclease activity, with fidelity 83 times that of Taq DNA polymerase and 9 times that of ordinary Pfu DNA polymerase. The enzyme solution contains two monoclonal antibodies that inhibit polymerase activity and 3'→5' exonuclease activity at room temperature, enabling simple and highly specific Hot Start PCR, significantly improving the detection rate and specificity of the amplified products. The enzyme solution contains extension factors, enabling the enzyme to amplify long fragments, with the length of the amplified target fragment reaching up to 13 kb. This product is equipped with an optimized buffer, making the enzyme suitable for the amplification of complex templates. The amplified products have blunt ends.

Specifications

Name	Cat. No.	Q10153-1	Q10153-2	Q10153-3	Q10153-4
QKGEN® High-Fidelity DNA Polymerase	Size	250U	500U	1000U	10000U

Components

Components No.	Name	Q10153-1	Q10153-2	Q10153-3	Q10153-4
10153-A	QKGEN® High-Fidelity DNA Polymerase (1 U/μL)	250 μL	500 μL	500 μL×2	500μL×4
10153-B	2× Plus PCR buffer (Contains Mg2+, dNTPs)	6.25 mL	12.5 mL	25 mL	50 mL
10153-C	6 × DNA Loading Buffer	3.0 mL	6.0 mL	12 mL	24 mL

Storage

Transport with ice packs.This product should be stored at -25~-15℃ for 12 months.

Application

Gene cloning; complex DNA template amplification; high-throughput library construction.

Recommended PCR reaction system

1.PCR reaction system

Components	Volume(μL)	Final concentration
2× Plus PCR buffer(Contains Mg2+, dNTPs)	25	1×
QKGEN® High-Fidelity DNA Polymerase(1 U/μL)	1	1U
Forward Primer(10 μmol/L)	2	0.4 μmol/L
Reverse Primer(10 μmol/L)	2	0.4 μmol/L
DNA	X	
ddH_2O	up to 50	

[Note]:

a) Reagent use: Thaw and mix thoroughly before use..

b) Mg^{2+} final concentration: The final concentration of the system is 2 mM. If special requirements exist, 50 mM $MgCl_2$ can be used, with increments of 0.2–0.5 mM.

c) High GC templates: Adding DMSO to a final concentration of 3% in the system may enhance amplification.

d) Recommended usage amounts for different templates (25 μL reaction system):

Template Types	Amplified fragment 1 kb-10 kb
genomic DNA	50ng - 200ng
Plasmid or viral DNA	10pg - 20ng
cDNA	1 - 2.5μL (no more than 1/10 of the total PCR reaction volume)

2.Reaction program

There are three programs to choose from, and the two-step program is preferred.

Two-step procedure (preferred):

Cycle step	Temp.	Time	Cycles
Pre-denaturation	98°C	3 min	1
Transgender	98°C	10 sec	30-35
Extension	68°C	30 sec / kb	
Extended reach	72°C	5 min	1

Three-step procedure (conventional procedure):

Cycle step	Temp.	Time	Cycles
Pre-denaturation [1]	98°C	3 min	1
Transgender	98°C	10 sec	30-35
Annealing [2]	60°C	20 sec	
Extension [3]	72°C	30 sec / kb	
Extended reach	72°C	5 min	1

Gradient temperature annealing program (recommended for genes difficult to amplify):

Cycle step	Temp.	Time	Cycles
Pre-denaturation	98°C	3 min	1
Transgender	98°C	10 sec	15, each cycle reduces the temperature by 1°C
Gradient annealing	70-55°C	20 sec	
Extension	72°C	30 sec / kb	
Transgender	98°C	10 sec	20
Normal annealing	55°C	20 sec	
Extension	72°C	30 sec / kb	
Extended reach	72°C	5 min	

*Characteristics of different amplification programs:

Program type	two-step method	Three-step method	Gradient annealing
Speed	Fastest	medium	slow
Specificity	high	medium	high
PCR output	medium	Highest	medium
Detection rate	high	medium	high

[Note]:

1.Pre-denaturation temperature and time: Recommended temperature: 98°C, time: 3 min, 5-10 min for high GC content templates.

2.Annealing temperature and time: Recommended temperature: 60°C, and a temperature gradient can be set to find the optimal temperature for index annealing as needed. The recommended annealing time is set to 20 sec, which can be adjusted within 10-30 sec. Annealing time that is too long may cause the amplification product to be diffuse on the gel.

3.Extension temperature and time: Recommended temperature: 72°C. Time: 30 sec/kb, and for complex templates, it can be extended to 60 sec/kb according to actual conditions.

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!