HB220429

Hieff Canace™ Plus High-Fidelity DNA Polymerase

Product Information

Product Name	Cat#	Size
Hieff Canace™ Plus High-Fidelity DNA Polymerase	10153ES60	100 U
	10153ES76	500 U
	10153ES80	1000 U

Product Description

Hieff Canace™ Plus High-Fidelity DNA Polymerase is based on the *Pyroccus Furiosis* DNA Polymerase, genetically engineered. The enzyme has a 5'→3' DNA polymerase activity and a 3'→5' exonuclease activity, and its fidelity is 83 times that of Taq DNA polymerase and 9 times that of ordinary *Pfu* DNA polymerase. Two monoclonal antibodies at room temperature that inhibit polymerase activity and 3'→5' exonuclease activity were added to the enzyme solution, which can easily perform highly specific Hot Start PCR, greatly improving the detection rate of amplification and the specificity of the product. The addition of an extension factor to the enzyme solution gives the enzyme the ability to amplify long fragments, and the length of the amplification of the fragment of interest can be up to 13 kb. This product is equipped with an optima buffer that makes the enzyme suitable for amplification of complex templates. It generates blunt ends in the amplification products.

Package Information

Component Number	Components	Cat#/Size		
		10153ES60 (100 U)	10153ES76 (500 U)	10153ES80 (1,000 U)
10153-A	Hieff Canace™ Plus High-Fidelity DNA Polymerase (1 U/μL)	100 μL	500 μL	2 × 500 μL
10153-B	2× Canace™ Plus PCR buffer（include Mg^{2+}，dNTPs）	3 × 1 mL	15 × 1 mL	30 × 1 mL
10153-C	6× DNA Loading Buffer	1 mL	6 × 1 ml	12 × 1 ml

Applications

Gene cloning; amplification of complex templates DNA; high-throughput library building.

Unit Definition

The activity of ingesting 10 nmol of deoxyribonucleotide as an acidic insoluble with activated salmon sperm DNA as a template/primer at 74°C, within 30 min is defined as 1 U.

Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C for one year. Please avoid repeated freeze-thaw.

Cautions

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. This product is for research use **ONLY**!

Reaction System

Component	Volume (μL)	Final Concentration

ddH_2O	to 50	-
2× Canace™ Plus PCR buffer (include Mg^{2+}, dNTPs)	25	1×
Template DNA	varies	-
Forward Primer (10 μmol/L)	2	0.4 μmol/L
Reverse Primer (10 μmol/L)	2	0.4 μmol/L
Hieff Canace™ Plus High-Fidelity DNA Polymerase (1 U/μL)	1	1 U/50 μL

[Note]: 1.Gently vortex and briefly centrifuge all solutions after thawing.

2.The final concentration of Mg^{2+} is 2 mM. But it can be varied in a range of 0.2–0.5 mM, if needed.

3. Add 3% DMSO as a PCR additive, which aids in the denaturing of templates with high GC contents.

4. Recommended template dosage (25 μL volume):

Templates	Amplify fragments from 1 kb to 10 kb
genomic DNA	50 ng-200 ng
plasmid or viral DNA	10 pg-20 ng
cDNA	1-2.5 μL (Do not exceed 10% of the final PCR reaction volume)

PCR Protocol

Two-Step Protocol (priority protocol)

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	3 min	1
Denaturation	98°C	10 sec	30-35
Extension	68°C	30 sec/kb	
Final extension	72°C	5 min	1

Three-Step Protocol (regular protocol)

Cycle step	Temp.	Time	Cycles
Initial denaturation[1]	98°C	3 min	1
Denaturation	98°C	10 sec	
Annealing[2]	60°C	20 sec	30-35
Extension[3]	72°C	30 sec/kb	
Final extension	72°C	5 min	1

Annealing Gradient Protocol (complexity template)

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	3 min	1
Denaturation	98°C	10 sec	15, -1°C/cycle
Gradient annealing	70-55°C	20 sec	
Extension	72°C	30 sec/kb	
Denaturation	98°C	10 sec	20
Annealing	55°C	20 sec	
Extension	72°C	30 sec/kb	
Final extension	72°C	5 min	1

*Features under different amplification protocol

Protocol	Two-Step	Three-step	Gradient annealing
Speed	fast	medium	slow
Specificity	high	medium	high
PCR yield	medium	high	medium
Detection rate	high	medium	high

[Note]: 1. Initial denaturation: We recommend a 3-min initial denaturation at 98°C for most templates, recommend5-10 min for GC-rich template.

2. Annealing: Recommended temperature: 60°C, you can also set a temperature gradient to touch the optimal temperature of index annealing as needed. The recommended annealing time is set to 20 sec, which can be adjusted within 10-30 sec. Annealing time too long may cause the amplification product to spread out on the gel.

3. Extension: Recommended temperature: 72°C. Time: 30 sec/kb, complex templates can be extended to 60 sec/kb depending on the actual situation.