HotStart Taq DNA Polymerase

Lot. No.: 1401B **Exp. date: 2016-02**

Kit Content

Cat. No.	*HotStart Taq* DNA Polymerase	10 x Reaction Buffer with $MgCl_2$	Dilution Buffer	dNTP Mixture
E-2017	250 units	0.5 ml	0.5 ml	0.5 ml
E-2017-1	1,000 units	4 x 0.5 ml	4 x 0.5 ml	4 x 0.5 ml
E-2017-2	500 units	2 x 0.5 ml	2 x 0.5 ml	2 x 0.5 ml
E-2017-3	250 units	0.5 ml	0.5 ml	-
E-2017-4	1,000 units	4 x 0.5 ml	4 x 0.5 ml	-

Specifications

Taq DNA Polymerase	
Concentration	5 units/µl
5'→3' exonuclease activity	Yes
3'→5' exonuclease activity	No
3' A overhang	Yes
Nuclease contamination	No
Extension rate	3–10 kb/minute depending on template complexity

Buffer and Reagents

10 x Reaction Buffer with $MgCl_2$: 100 mM Tris-HCl, 450 mM KCl, 15 mM $MgCl_2$, pH 9.0

Dilution Buffer : 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 50 % glycerol, pH 8.0

dNTP Mixture : 10 mM (2.5 mM each dNTP)

Storage Conditions

HotStart Taq DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at –20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Applications

Routine PCR, primer extension, TA cloning, gene sequencing, SNP, multiplex PCR, Real-Time PCR

Description

HotStart Taq DNA Polymerase is designed to perform Hotstart PCR. The *HotStart Taq* DNA polymerase is inhibited at lower temperature, but is fully activated at temperature above 70°C. This prevents the formation of mis-primed products and primer dimers during the reaction setup process, resulting in improved PCR specificity.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72°C.

Quality Assurance

Nuclease activity is not detected after incubation of 1 µg of substrate DNA - supercoiled plasmid and Lambda/*Hin*d III DNA with 5 units of *HotStart Taq* DNA Polymerase in 50 µl reaction volume with the supplied reaction buffer for 18 hours at 37°C and 70°C.

Protocol

1. Thaw 10 x *HotStart Taq* Reaction Buffer, dNTP mix, primer solutions and template DNA.
2. Prepare a reaction mixture.

Component	20 µl reaction	50 µl reaction
Template*	Variable	Variable
Forward primer (10 pmole/µl)	1 - 2 µl	2.5 - 5 µl
Reverse primer (10 pmole/µl)	1 - 2 µl	2.5 - 5 µl
10X Reaction Buffer	2 µl	5 µl
10 mM dNTP (2.5mM each)	(Variable volume) or 2 µl	(Variable volume) or 5 µl
HotStart Taq DNA polymerase (5 units/µl)	0.5 - 1.0 unit	1 - 2.5 unit
PCR grade water	Variable	Variable

* Recommended amounts of template: Plasmid and lambda DNA: > 1 pg, Bacterial genomic DNA: > 10 pg, Human genomic DNA: > 1 ng

3. Mix the reaction mixture thoroughly and dispense appropriate volumes into PCR tubes.
4. Add template DNA to individual PCR tubes.

HotStart Taq DNA Polymerase

5. Perform the reaction under the following conditions.

For Standard PCR (3-step)

Step	Temperature	Time	Cycles
Pre-denaturation	94°C	15 min	1 cycle
Denaturation	94°C	0.5 -1 min	25-35 cycles
Annealing	AT* °C	0.5 -1 min	
Extension	72 °C	1 min/kb	
Final extension	72 °C	Optional. Normally 5-10 min	1 cycle

* Annealing temperature (approximately 5°C below Tm of primers)

6. Maintain the reaction at 4°C after the completion of amplification. It is recommended to store samples at -20°C until use. Analyze the PCR products by agarose gel electrophoresis.

Experimental Data

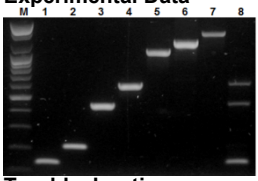

Figure 1. *HotStart Taq* DNA polymerase has high amplification efficiency and specificity. p53 gene was amplified with different sets of primers from human genomic DNA. Each lane 1-7 represents results of single PCR amplification and lane 8 represents results of multiplex PCR amplification with 3 sets of primers.

Lane M: 100 bp DNA Ladder (Cat. No. D-1030, Bioneer)

Lane 1: 139 bp
Lane 2: 211 bp
Lane 3: 447 bp
Lane 4: 618 bp
Lane 5: 1,082 bp
Lane 6: 1,296 bp
Lane 7: 1,561 bp
Lane 8: Multiplex PCR (139 bp, 447 bp, 618 bp)

Troubleshooting

Possible Cause	Recommendation
No product or low yield	
Insufficient template	Increase the amount of template used in PCR. High quality templates are essential for amplification of long targets. Check the purity of template or repeat purification of template.
Enzyme concentration is too low	If necessary, increase the amount of enzyme in 0.5 U steps.
$MgCl_2$ concentration is too low	Increase the amount of $MgCl_2$ concentration in steps.
Primer design is not optimal	Design alternative primers.
Cycle conditions are not optimal	Reduce the annealing temperature. Increase the number of cycles.
Amplification of GC-rich genes	Add 0.5-1 M Betaine or 2-8 % DMSO.
Product is multi-banded or smeared	
Annealing temperature is too low	Increase annealing temperature according to primer length.
Incorrect extension time	Adjust the time of the extension step according to the size of the expected PCR product.
Primer design is not optimal	Design alternative primers.
Problems with template	Check the concentration, storage conditions, and quality of template.
Too many cycles	Reduce the number of cycles.
Incorrect enzyme concentration	Reduce the amount of enzyme in decrements of 0.5 U.
Products in negative control experiments	
Carry-over contamination	Set up PCR reactions in an area separate from that used for PCR product analysis.

Related Products

Cat. No.	Products
K-2611	*AccuPower PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube, 96 tubes
K-2612	*AccuPower PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube, 480 tubes
K-2613	*AccuPower PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps, 50 µl reaction/tube, 96 tubes
K-2614	*AccuPower PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction/tube, 480 tubes
D-3001	10 mM dNTP Mixture (1.0 ml, 2.5 mM each dNTP)