UltraHiPF® DNA Polymerase Kit
User Manual

Cat. No. **PC018 (100U)**
Cat. No. **PC019 (5×100U)**

User Manual

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UltraHiPF® DNA Polymerase Kit

I. Description

UltraHiPF® DNA Polymerase is a heat-resistant ultra-fidelity DNA polymerase with 5'- 3' polymerase activity and 3'- 5' exonuclease activity. The polymerase can amplify uracil- and hypoxanthine- containing templates, GC- or AT- rich templates, genomes as low as one copy, and DNA fragments of more than 12 kb from different species. UltraHiPF® DNA Polymerase can specifically amplify 2 kb fragments at 1sec denaturation, annealing, and elongation, and can expand complex templates at 15-30 sec/kb. With Sanger sequencing, the mutation rate of this enzyme is 55 times lower than that of Taq DNA polymerase. The PCR product obtained by using the product has the Blunt-end.

■ Advantages

✧ 55X higher fidelity than Taq
✧ Robust reactions - maximal success with minimal optimization
✧ Most templates can be expanded up to 0.5 sec/kb
✧ Superior performance for a broad range of amplicons

■ Quality Guarantee

No endonuclease or RNase activity was detected. Purity > 99%.

II. Contents and Storage

Store all components at -20℃ (stable for at least 12 months). Avoid repeated freezing/ thawing.

Cat. No.	Contents	Part No.	Quantity
PC018	UltraHiPF® DNA Polymerase (1U/μl)	PC018-01	100μl
	5× HiPF Buffer (with Mg^{2+})	PC018-02	1ml
	5× Enhancer	PC018-03	1ml
	25mM $MgSO_4$	PC018-04	500μl
	10mM dNTP	PC018-05	100μl
	ddH_2O (DNase/RNase Free)	QP006-07	1ml
PC019	UltraHiPF® DNA Polymerase (1U/μl)	PC018-01	100μl×5
	5× HiPF Buffer (with Mg^{2+})	PC018-02	1ml×5
	5× Enhancer	PC018-03	1ml×5
	25mM $MgSO_4$	PC018-04	500μl×5
	10mM dNTP	PC018-05	100μl×5
	ddH_2O (DNase/RNase Free)	QP006-07	1ml×5

IV. Procedures

Ordinary PCR

1. Thaw all the reagents needed for PCR in the UltraHiPF® DNA Polymerase Kit. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Pre-heat the PCR instrument.
3. Prepare the reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
5× HiPF Buffer (with Mg^{2+})	10 μl	1×[a]
5× Enhancer	10 μl	variable[b]
Forward Primer 10 μM	2 μl	0.2~1 μM
Reverse Primer 10 μM	2 μl	0.2~1 μM
10mM dNTP	1 μl	0.2 mM
Template	Optional	1pg~10ng
UltraHiPF™ DNA polymerse (1U/μl)	1 μl	0.5~2U[c]
ddH2O	Up to 50 μl	

4. The following method for programming the reverse transcription reaction is recommended:

Temperature	Time	Cycles
98℃[f]	1~3 min[d]	1
98℃	10~15 sec[d]	25-35 cycles
Tm ±3℃[e]	10~30 sec[e]	
72℃	15~30 sec/kb	
72℃	7 min	1
4℃ hold		1

Note:

a. The final concentration of the system contains 2 mM Mg^{2+}.If it is necessary to adjust Mg^{2+}concentration,use the 25 mM MgSO4 provided in the kit to adjust the optimal concentration of Mg^{2+} at an interval of 0.2~0.5 mM. The final concentration of Mg^{2+} is generally not more than 3mM.

b. For difficult templates, adjust 5× Enhancer in the range of 0 to 20 μl as required.

c. The range of concentration of UltraHiPF® DNA Polymeras is from 0.5 to 2U/50 μl. Since the enzyme has 3 '-5' exonuclease activity, the enzyme should be added last when configuring the reaction system, and the reaction should be carried out immediately. If the additionalA of TA cloning is required, the PCR products must be purified.

d. The pre-denaturation time of most templates is 1 min, and the denaturation time of each cycle is 10 sec. For difficult templates, appropriately increase the pre-denaturation time to 3min, and the denaturation time of each cycle is 15 sec.

e. To obtain better specificity, the annealing temperature should be as high as possible to the primer Tm value, within the range of +3 ℃. A long annealing time may result in the dispersion of the amplified products, so the recommended annealing time is 10 sec. For difficult templates, the annealing time can be adjusted between 10 to 30 sec.

f. The PCR reaction system should be prepared on the ice and directly placed on the PCR apparatus preheated to 80℃ for reaction, which can enhance the specificity of PCR reaction and reduce the non-specific amplification.

Long PCR

UltraHiPF® DNA Polymerase has excellent high specificity and long fragment amplification. If more than 12kb of fragments need to be amplified, it is recommended to increase the amount of template (100ng/50μl for the genome is recommended), reduce the denaturation temperature in the cycle, increase the Tm value of the primer (no more than 68 ℃), and adjust the extension temperature to 68 ℃. If necessary, adjust the optimal concentration of Mg^{2+} at an interval of 0.2 to 0.5 mM.

Temperature	Time	Cycles
94℃	3 min	1
94℃	10~15 sec	30-35 cycles
Tm	30 sec	
68℃	30~60 sec/kb	
68℃	7 min	1
4℃ hold		1

High GC fragment PCR

UltraHiPF® DNA Polymerase can amplify high GC fragments with high specificity and high yield that cannot be amplified by conventional polymerases. The **common PCR** system and procedures used in this user manual can solve most amplification experiments perfectly. If necessary, consider adjusting the amount of 5× Enhancer to 10 to 20 μl.

Low GC fragment PCR

For the amplification of low GC fragments, it is recommended to adjust the denaturation time to 2 sec, the Tm value of the primer to 65℃, the extension temperature to 65℃~68℃, and use "sub-cycle" to increase the amplification yield. If necessary, consider adjusting the amount of 5× Enhancer to 0 to 10 μl.

Temperature	Time	Cycles	Cycles
94℃	2~3 min	1	
94℃	2 sec	1	30-35 cycles
68℃[①]	15~30 sec[②]	4 cycles	
63℃[①]	15~30 sec[②]		
68℃	variable[③]	1	
68℃	7 min	1	
4℃ hold		1	

Note:

a. If the primer Tm value is 65℃, "sub-cycle" is performed at 68℃ and 63℃. If the primer Tm value is 62℃,"sub-cycle" is performed at 65℃ and 60℃. If the Tm value is too low, it is not conducive to the amplification of low GC fragments.

b. The "sub-cycle" time is 15~30 sec. If the amplification length is less than 2kb, 15 sec is sufficient.

c. Since the "sub-cycle" is already cryogenic amplification, there is no need to add an additional 68℃ extension for fragments below 4kb. If the fragment is larger than 4kb, set the additional 68℃ extension time at 30~60 sec/ (template length minus 4kb) kb.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of UltraHiPF® DNA Polymerase Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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