

2× UltraHiPF® PCR Mix User Manual

2× UltraHiPF® PCR Mix

Cat. No: PC033 (1 ml)

Cat. No: PC034 (5 ml)

User Manual

GeneCopoeia, Inc.
9620 Medical Center Drive, #101 Rockville, MD 20850
USA

301-762-0888

inquiry@genecopoeia.com

www.genecopoeia.com

2× UltraHiPF® PCR Mix

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I. Description

2× UltraHiPF® PCR Mix is a 2× premixed solution containing UltraHiPF® DNA Polymerase, dNTPs, and all other components required for PCR, excluding DNA templates and primers. The premixed solution saves time and reduces contamination by reducing the number of pipetting steps required to prepare the PCR reaction system.

UltraHiPF® DNA Polymerase is a heat-stable, 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, low copy genomes, and DNA fragments greater than 12 kb in length from various species. The enzyme has a 55-fold lower mutation rate than Taq DNA polymerase by Sanger sequencing. The PCR products obtained with the product have blunt ends.

■ Advantages

- ✧ 55X higher fidelity than Taq
- ✧ Robust reactions - maximal success with minimal optimization
- ✧ Superior performance for a broad range of amplicons

■ Quality Guarantee

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

II. Contents and Storage

Cat. No.	Contents	Quantity	Store
PC033	2× UltraHiPF® PCR Mix	1ml	Store all components at -20°C (stable for at least 12 months).
PC034	2× UltraHiPF® PCR Mix	1ml×5	Store all components at -20°C (stable for at least 12 months).

Avoid repeated freezing/ thawing.

IV. Procedures

Ordinary PCR

1. Thaw the 2× UltraHiPF® PCR Mix. 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
2× UltraHiPF® PCR Mix	12.5µl	1x
Forward Primer 10 µM	1µl	0.4µM
Reverse Primer 10 µM	1µl	0.4µM
Template	Optional	1pg~10ng (plasmid) 10~250ng (genome)
ddH ₂ O	Up to 25 µl ^a	

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

Temperature	Time	Cycles
98°C ^b	1~3 min ^c	1
98°C	10~15 sec ^c	25-35 cycles
T _m ±3°C ^d	10~30 sec ^d	
72°C	15~30 sec/kb	
72°C	7 min	1
4°C hold		1

Note:

- a. The reaction system can be increased to 50µl with the final concentration of each component unchanged.
- b. The PCR reaction system should be prepared on the ice and directly placed on the PCR apparatus preheated to 80°C for reaction, which can enhance the specificity of PCR reaction and reduce the non-specific amplification.
- c. 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.
- d. To obtain better specificity, the annealing temperature should be as high as possible to the primer T_m value, within the range of +3 °C. 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.

Long PCR

2× UltraHiPF® PCR Mix 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 T_m value of the primer (no more than 68 °C), and adjust the extension temperature to 68 °C.

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

High GC fragment PCR

2× UltraHiPF® PCR Mix 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.

Low GC fragment PCR

For the amplification of low GC fragments, it is recommended to adjust the denaturation time to 2 sec, the T_m value of the primer to 65 °C, the extension temperature to 65 °C~68 °C, and use "sub-cycle" to increase the amplification yield.

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:

- If the primer T_m value is 65 °C, "sub-cycle" is performed at 68 °C and 63 °C. If the primer T_m value is 62 °C, "sub-cycle" is performed at 65 °C and 60 °C. If the T_m value is too low, it is not conducive to the amplification of low GC fragments.
- The "sub-cycle" time is 15~30 sec. If the amplification length is less than 2kb, 15 sec is sufficient.
- Since the "sub-cycle" is already cryogenic amplification, there is no need to add an additional 68 °C extension for fragments below 4kb. If the fragment is larger than 4kb, set the additional 68 °C 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 2× UltraHiPF® PCR Mix (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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9620 Medical Center Drive, #101, Rockville, MD 20850

Tel: 301-762-0888 Fax: 301-762-3888,

Email: inquiry@genecopoeia.com

Web: www.genecopoeia.com