TIANGEN®

Ultra HiFidelity PCR Kit

High fidelity, high specificity and high efficiency hot-start PCR premix

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Ultra HiFidelity PCR Kit

Cat. no. 4992970/4992971/4992978

Kit Contents

Contents	4992970	4992971	4992978
2×UltraHiFi Mix (with dye)	1 ml	5×1 ml	5×5×1 ml
PCR Enhancer	500 μl	500 μl	5×500 μl
ddH_2O	1 ml	5 ml	5×5 ml
Handbook	1	1	1

Storage condition

Please store the kit at -30~-15°C for up to one year and avoid repeated freezing and thawing.

Product Introduction

Ultra HiFidelity PCR Kit is a new HiFi PCR amplification pre-mixed liquor and is applicable to PCR-related clone and detection. The Ultra HiFi DNA Polymerase in the amplification pre-mixed liquor is a new fast HiFi DNA polymerase developed by the directional molecular evolution technology.

It enhances the affinity of DNA polymerase to the templates, improves the amplification speed and elongation ability and increases the PCR success rate and product amount. Moreover, the polymerase has high-quality 3'-5' proofreading activity. Its fidelity reaches the level of mainstream products on the market, ensuring the authenticity of gene and library amplification process. In addition, the DNA polymerase in the product has the Hot Start function, which can effectively control the non-specific amplification and enzyme activity loss under low temperature, thus ensuring the specificity and stability of PCR amplification.

The product is a one-tube pre-mix liquor and is composed of hot start Ultra HiFi DNA Polymerase, ultra-pure dNTPs, $MgCl_2$, reaction buffer and other components required for the PCR reaction. Just adding the templates and primers in the product, the PCR amplification reaction can be easily operated. Besides, the product has integrated PCR Enhancer components, which respectively improves the resistant ability and adaptive ability of Ultra HiFidelity PCR Kit to PCR reaction inhibitors and to templates with different GC content, so that the kit can be used when amplifying advanced product with complex structure and PCR system with a high PCR inhibitor content.

The PCR product of the product is the blunt end, which can be used for gene clone after being treated with A and then conjugated to T carrier or directly being treated with blunt end cloning vector, such as TIANGEN Lethal Based Fast Cloning Kit (Cat#: 4992815).

Product Features

High fidelity: 50 times higher fidelity than *Taq* polymerase.

Ultra specificity: Real hot start, strong specificity.

Fast amplification: Amplification speed of 10-15 sec/kb.

Strong extension: Capable of amplifying up to 20 kb of DNA fragments

High sensitivity: Template amount can be lower to 1 pg.

Low preference: Even amplification efficiency for different types of DNA fragments.

Application

It can be used for DNA high fidelity and fast amplification, such as analyses of gene expression and cloning, gene site-directed mutation and genome point mutation (SNP).

I. Preparation of PCR reaction solution:

1. Take 2×UltraHiFi Mix (with dye), primer, template and ddH_2O for melting under room temperature (15-30°C), fully mix them, and put them on ice for preparation after simple centrifugation.
2. Prepare the PCR reaction system according to the following system:

Components	Volume	Reaction concentration
DNA Template	Variable **	-
Primer F* (10 μM)	1.25 μl	0.25 μM
Primer R* (10 μM)	1.25 μl	0.25 μM
2×UltraHiFi Mix (with dye)	25 μl	1×
PCR Enhancer*** (if needed)	10 μl	1×
ddH_2O	To 50 μl	-

Notes: the reaction tubes should be put on ice during the whole process of preparing reaction system. for multiple samples, please calculate the total reagent volume and add 10% reagent on this basis to avoid the tip wall attachment loss in the process of sub-package and lead to the insufficient volume of reagent.

*When the final concentration of the primer is 0.25 μM, the amplification conditions are good in most systems. In case of lower amplification efficiency, improve the primer concentration in the PCR reaction system; properly reducing the primer concentration in the reaction system can improve the specificity of the PCR reaction. If necessary, conduct optimization selection between 0.2-1.0 μM.

** Please refer to the following table for the template DNA amount (50 μl PCR reaction system):

Template type	Range of template amount	Recommended template amount
Genome DNA	1-1000 ng	100-500 ng
Plasmid DNA	0.01-100 ng	1-10 ng
cDNA	1-200 ng	50-100 ng
λDNA	0.01-100 ng	1-10 ng

*** PCR Enhancer can respectively improve the resistant ability and adaptive ability of Ultra HiFidelity PCR Kit to PCR reaction inhibitors and to templates with different GC content. For PCR amplification of advanced templates with complex structure and templates with high GC content and inhibitor, in case of poor amplification, add PCR Enhancer in the PCR system.

3. Add template, primer and ddH_2O based on the recommended amount of template and primer in Step 2, load it on the machine for PCR reaction after complete mixing.

II. PCR reaction conditions:

1. When using 2×UltraHiFi Mix (with dye) for amplification reaction, three-step approach should prevail.

   Notes: when carrying out amplification by three-step approach, the amplification speed should be set to 10-15 sec/kb; For templates with the DNA length ≥10 kb or complex templates, the amplification time can be extended to 30 sec/kb. The following reaction procedure is for reference only, customers can change and adjust it according to the actual condition

   The three-step approach is as follows:

Steps	Temperature	Time	Cycle threshold
1	94°C	2 min	1
2	98°C	10 sec	35
3	60°C*	30 sec	
4	68°C	10-15 sec/kb	
5	68°C	5 min	1
6	4°C	Hold	1

* The amplification conditions are good in most systems with the annealing temperature of 60°C. In case of lower specificity of the PCR reaction, properly increase the annealing temperature within the scope of 55-68°C; If the Tm of the primer is lower than 63°C, keep the annealing temperature be the same as Tm value.

2. In case of any heteroid bands or dispersion of the amplification product, please adopt the two-step approach or step down PCR. The two-step approach is as follows:

Steps	Temperature	Time	Cycle threshold
1	94°C	2 min	1
2	98°C	10 sec	35
3	68°C	10-15 sec/kb	
4	68°C	5 min	1
5	4°C	Hold	1

3. Detection results: After the reaction, 5 μl of the reaction product was detected by agarose gel electrophoresis.

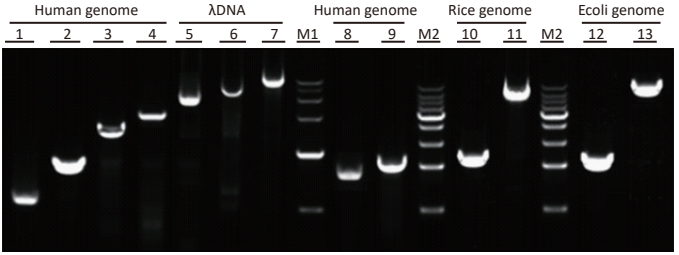
Notes: The electrophoresis indicator has been added to the 2×UltraHiFi Mix (with dye), no additional Loading Buffer is needed.

III. Types of amplification templates and examples of long fragment amplification:

1. The applicable scope and amplification length of the recorded samples are shown in the table below:

Template type	Recorded long fragment amplification
Human genome DNA	-8 kb
Rat genome DNA	-8 kb
Rice genome DNA	-8 kb
Wheat genome DNA	-8 kb
Corn genome DNA	-8 kb
Colibacillus genome DNA	-8 kb
cDNA	-6 kb
λDNA	-15 kb

2. Specific amplification effect examples:



1: length of 1kb; 2: length of 2kb; 3: length of 4kb; 4: length of 6kb; 5: length of 8kb;
6: length of 10kb; 7: length of 15 kb; 8: length of 1.5kb (GC content of 61.5%);
9: length of 1.9 kb (GC content of 70.4%);
10: length of 2.2kb; 11: length of 8 kb; 12: length of 2.1 kb; 13: length of 8 kb;

FAQs

Possible causes	Possible causes	Solution
No amplification product or few amplification products	Improper cyclic condition	The amplification time can be extended to 15-30 sec/ kb.
		Increase 2-5 cycles
		Use the Step down PCR (effective for amplified fragments above 8 kb)
	The quality and quantity of template DNA cannot meet the requirements	Increase the template amount
		Reduce the template amount (lower the inhibiting effect of excessive template or reduce the interference of PCR inhibitor in impure templates)
		Try to use the purified templates
		RNA in templates should be completely removed
	Primer problems	Improper primer concentration, for the longer amplification fragment, try to select the relatively lower primer concentration (0.2-0.3 μM); In case of lower template concentration, try to use the relatively higher primer concentration (0.3-0.5 μM)
		Try to use the freshly prepared primers
		Improper primer design, re-optimize primers
Have heteroid bands or dispersion	Improper cyclic condition	Increase the annealing temperature or adopt two-step approach
		Use Step down PCR
		Reduce 2-5 cycles
	Primers degradation or improper design	Re-prepare it or design primer (properly increasing primer length can improve the specificity between primers and templates)
	Excessive template	Add templates according to the recommended template amount in the handbook