Ultra HiFidelity PCR Kit II

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Ultra HiFidelity PCR Kit II

Cat. no. GKP213

Kit Contents

Contents	GKP213-01	GKP213-02
2×UltraHiFi Mix II	1 ml	5×1 ml
PCR Enhancer	500 μl	500 μl
6×DNA Loading Buffer (Blue)	1 ml	2×1 ml
ddH_2O	1 ml	5 ml

Storage

This product requires dry ice for transportation.

After receiving this product, please store it at -30~-15°C immediately. Shelf life is 24 months.

Introduction

This product is a new high-fidelity PCR amplification premix. Suitable for PCR-related cloning and detection.

The HiFi DNA Polymerase in the amplification premix is a new type of ultra-strong and high-fidelity DNA polymerase developed through directed molecular evolution technology. The modification enhances the 3'- 5' exonuclease activity (Proofreading activity) of the enzyme, which improves the fidelity of the DNA amplification process; at the same time, it also enhances the affinity of the enzyme to the template, which improves the enzyme's sensitivity and elongation ability. It enhances the amplification efficiency and the success rate of subsequent cloning. In addition, the DNA polymerase in this product also 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.

This product is a easy to operate one-tube premixed Mix format, adding the template and primers when the experiment. In addition, the PCR Enhancer component is integrated into the product, which can improve the tolerance of Ultra HiFidelity PCR Kit II to PCR inhibitors and adaptability to templates with different GC contents, so it can be added when amplifying products with complex high-level structures and PCR systems with high PCR inhibitor contents.

The PCR products are blunt-end and can be used directly for gene cloning using TIANGEN blunt-end cloning vectors (Cat.no GVT205/GVT206) or seamless cloning technology (Cat.no GVI201/GVI202).

Features

Strong extension power: Amplification rate up to 5 sec/kb, with amplification products up to 20 kb.

Good generalizability: Capable of recognizing DNA fragments of different species with GC content of 30%~80%.

High sensitivity: The amount of genomic template can be as low as 1 ng.

Easy to use: Simply add the template and primers to easily prepare the system. The PCR product is blunt-end and can be used directly for seamless cloning after purification.

Application

It is used for high-fidelity amplification of DNA, such as gene expression cloning, gene point mutation, and analysis of genomic point mutations (SNP).

Protocol

1.Thaw the template DNA on ice; thaw 2× UltraHiFi Mix II, PCR Enhancer and ddH_2O at room temperature (15-30°C), and set aside on ice quickly after thawing. Mix 2×UltraHiFi Mix II with vortex oscillation before use.

2.Prepare the reaction system as shown in the table below.

Component	50 μl reaction system addition	Reaction concentration
DNA Template	Variable[*2]	-
Primer F[*1](10 μM)	1.5 μl	0.3 μM
Primer R[*1](10 μM)	1.5 μl	0.3 μM
2×UltraHiFi Mix II	25 μl	1×
ddH_2O	To 50 μl	-

Note:

1.PCR Enhancer (5× concentration) is included in the kit, which is not necessary to be added in general PCR reactions. When the amplification region contains complex high-level structures or the GC content is more than 65% and the amplification effect is not good, you can consider adding PCR Enhancer to the PCR system (set the concentration of the reaction between 0-1×, i.e., add 0-10 μl PCR Enhancer to 50 μl of the system) to enhance the amplification effect.

2.Keep the reaction tubes on ice throughout the preparation of the reaction system. For multiple samples, calculate the total volume of reagents required and add an additional 10% to this to avoid insufficient volume of reagents due to loss of wall hanging of the gun tip during dispensing.

[*1] A final primer concentration of 0.3 μM gives good amplification results in most systems. If the amplification efficiency is not high, the primer concentration in the PCR reaction system can be increased; if the primer concentration in the PCR reaction system is appropriately reduced, the specificity of the PCR reaction can be increased. If necessary, the selection can be optimized between 0.2-1.0 μM.

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

Template type	Template amounts	Recommended template amounts
Genomic DNA	1-1000 ng	100-500 ng
Plasmid DNA	0.01-100 ng	0.1-10 ng
cDNA	1-200 ng	50-100 ng
λDNA	0.01-100 ng	1-10 ng

3. Set up the reaction program as shown in the table below.

Reaction temperature	Reaction time	Number of cycles	Note
98°C	1 min	1	Pre-denaturation
98°C	10 sec	35	PCR Cycle Steps
60°C[*3]	20 sec		
72°C	5-10 sec/kb[*4]		
72°C	5 min	1	Additional extension
4°C	Holding	1	Low temperature storage

[*3] Annealing at 60°C gives good amplification results in most systems. When the specificity of PCR reaction is not high, the annealing temperature can be adjusted within the range of 55-68°C; if the Tm value of the primer is less than 63°C, the annealing temperature can be set according to the Tm value.

[*4] When the amplification product is less than 10 kb, the extension speed can be selected 5 sec/kb; when the amplification product is greater than or equal to 10 kb, the extension speed should be selected 10 sec/kb.

Note: The above reaction program is for reference only, in actual situation, customers can change and adjust according to their own situation.

4. Experimental results are analyzed.

At the end of the reaction, 5 µl of the reaction product was taken and mixed with 6×DNA Loading Buffer (Blue) and then detected by agarose gel electrophoresis.

Important Notes

1.Mix 2×UltraHiFi Mix II well before use.

2.PCR Enhancer is not a necessary component of the PCR system and may be considered to be added when amplification is poor, non-specific and severe or when performing complex template amplification.