Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

HiFi Long PCR MasterMix

Product Number: PCM43R

Shipping and Storage

Store at -20°C for 2 years. For short-term use (within 1 month), it can be kept at 4°C.

Component

Component	PCM43R
HiFi Long PCR MasterMix	1mL

Description

This product contains HiFi Long DNA Polymerase, dNTPs, and optimized reaction buffer at a concentration of 2 ×. When using, simply add the template and primer, and add water until the final concentration of Mix is 1 ×. This product is a mixture of various ultra fidelity enzymes modified with advanced genetic engineering and extended factors, greatly improving amplification length, amplification speed, fidelity, and yield. This product is the preferred product for long fragment ultra fidelity rapid amplification. This product contains a red tracer dye and can be directly loaded for electrophoresis without the need for sample buffer; It can also be purified for subsequent operations such as enzyme digestion, ligation, fluorescence sequencing, etc. The PCR product is a flat end and does not require the addition of an A head. It can be directly cloned using the pTOPO Blunt flat end series vector.

Features

1. Fast amplification speed: The extension speed can reach 3-4kb/min, which is 6-8 times faster than PFU.
2. High amplification yield: Generally, the PCR product yield is 50% -100% higher than that of traditional pfu.
3. Excellent authenticity: The fidelity is over 54 times that of taq. Generally, randomly selecting a bacterium for sequencing is the correct and mutation free colony.
4. Long amplification length: Complex genomic DNA is suitable for amplifying products up to about 10kb, while simple genomic, plasmid, and phage DNA are suitable for amplifying products up to about 15kb.

Suggested PCR system setup

Component	25μL Reaction	50μL Reaction	Final Concentration
HiFi Long PCR MasterMix	12.5μL	25μL	1 ×
Forward Primer(10μM)	0.5μL	1μL	0.2μM
Reverse Primer(10μM)	0.5μL	1μL	0.2μM
Template DNA	as required	as required	
ddH_2O	up to 25μL	up to 50μL	

Reference template dosage (50μL reaction system):

Plasmid: 0.1-10ng; Bacterial genome: 10-100ng; Human genome: 50-150ng; cDNA: 1-5μL from RT reaction.

Suggested PCR cycle conditions

Step	Temperature	Time	Cycle Number
Initial denaturation	95°C	3 minutes	
Denaturation	95°C	10 seconds	30 cycles
Annealing	55°C	10-15 seconds	
Extension	72°C	15-25 seconds / kb	
Final Extension	72°C	2-5 minutes	
ddH_2O	4-8°C	Hold	

Note

1. This product has a fast amplification speed, and simple templates such as plasmids and genomes can be amplified in 15-20 seconds/kb; complex templates such as the human genome can be amplified in 25-30 seconds/kb.
2. If the main band is found to have blurred tails in electrophoresis, or if there is a smear band dragging down from the sample well in the lane, it generally indicates that the extension time is too long, and the gradient should be shortened (it is recommended to reduce the extension time by 10-15 seconds/kb) until satisfactory results are obtained.
3. For templates with high GC content, the pre denaturation and denaturation temperatures can be increased to 98℃. A9 has strong heat resistance, and 98℃ does not change its activity.
4. If the GC content of the amplification template is high or the amplification effect is poor due to the complexity of the template, DMSO can be added to the reaction mixture to a final concentration of 1% -8%, and the optimal concentration can be explored by increasing it in a 1% gradient. Alternatively, betaine can be added to a final concentration of 1.0-1.7 M. Touchdown PCR can be used.