Tinzyme Co., Limited

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

2×QuickPro PCR Master Mix

Product Number: PCM77

Shipping and Storage

Store at -20℃.

Components

Component	PCM77	PCM77
2×QuickPro PCR Master Mix	1 ml	5×1 ml
ddH_2O	1 ml	5×1 ml

Description

The 2×QuickPro PCR Master Mix contains QuickPro DNA Polymerase, unique elongation factors, dNTP, and a high-performance buffer system. PCR reactions can be performed by adding primers and templates. QuickPro DNA Polymerase is a carefully mutated, high fidelity DNA polymerase with 5 '-3' DNA polymerase activity and 3 '-5' exonuclease activity. It has strong amplification ability, fast extension speed, and good specificity.

This product is suitable for rapid PCR amplification of long and short fragments, with an extension speed of 1s/kb within 1kb, greatly saving PCR reaction time. At the same time, it has the unique advantages of certain fidelity and high yield, and its fidelity is about 80 times that of Taq DNA polymerase. The amplification product has a flat end and excellent amplification performance for crude samples.

Protocol

The following are examples of conventional PCR reaction systems and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

PCR reaction system

All operations should be carried out on ice. After the group is decomposed and frozen, please mix thoroughly. After use, please put it back at -20℃ for storage in a timely manner.

Component	25μLReaction system	Final Conc.
2×QuickPro PCR Master Mix	12.5μL	1×
Forward Primer,10μM	0.5-1μL	0.2-0.4μM
Reverse Primer,10μM	0.5-1μL	0.2-0.4μM
Template DNA	Appropriate amount	< 500ng
ddH_2O	up to 25μL	/

PCR reaction program

Step	Temperature	Time	Cycles
Pre denaturation	98℃	30s-3min[1)]	
Denaturation	98℃	10s	30-35Cycles[4)]
Annealing	Determine based on primer Tm[2)]	10s	
Extension	72℃	1-20s/kb[3)]	
Final extension	72℃	5min	

Note: 1) Pre denaturation: The pre denaturation time for templates such as plasmid DNA, lambda DNA, and simple genomic DNA can be set to 30s-1 minutes. For complex templates such as crude samples, high GC, and human genome, the pre denaturation time can be extended to 3 minutes.

2) Annealing: 2 × QuickPro PCR Master Mix contains high ion concentration, and the reaction annealing

temperature can be set 2-3°C higher than the theoretical primer Tm value. If the ideal amplification efficiency cannot be achieved, the annealing temperature can be gradient changed for optimization; When non-specific reactions occur, increase the annealing temperature appropriately.

3) Extension: Set the extension time according to the length of the target segment, as shown in the table below

Target fragment length	Suggest extending the time
< 1kb	1-2s/kb
1-5kb	2-5s/kb
5-10kb	5-10s/kb
> 10kb	10-20s/kb

4)Cycle number: The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient, or the number of cycles is too many, the probability of mismatch will increase. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.

Common problems and solutions

1. No amplification products or low concentration of amplification products
   - 1.1. The primer concentration can be appropriately increased;
   - 1.2. Set gradient annealing to find the appropriate annealing temperature;
   - 1.3. Appropriately increase the extension time or increase the number of PCR cycles;
   - 1.4. Adjust template usage or use high-purity templates.
2. More non-specific or diffuse bands
   - 2.1. Attempt to increase annealing temperature;
   - 2.2. Properly reduce the concentration of primers;
   - 2.3. Reduce the number of cycles;
   - 2.4. Adjust template usage or use high-purity templates;
   - 2.5. Optimize primer design.