Taq Pro Multiplex DNA Polymerase (High sensitivity)

PM201

Version 22.1

Product Description

Taq Pro Multiplex DNA Polymerase is a new generation of hot-start DNA polymerase based on antibody modification and upgraded to improve template affinity. The High sensitivity version has been carefully optimized for multiplex PCR with strong amplification ability and high detection sensitivity. With the optimal buffer system for multiplex PCR, it is compatible with a wide range of product GC content and primer Tm values. The vast majority of multiplex PCR assays can be performed at a universal annealing temperature of 60°C without additional optimization.

This product has **extremely high amplification efficiency**, and can simultaneously amplify target fragments in the range of 50 - 3,500 bp; it has **strong amplification ability**, and can carry out enrichment of several hundred or more amplicons; **excellent impurity tolerance**, highly resistant to a variety of inhibitors and impurity types, and compatible with direct amplification of whole blood, blood cards, etc. The reagents have good stability, wide applicability, and are compatible with a variety of detection scenarios.

Components

Components	PM201-01 (200 rxns/25 μl/rxn)	PM201-02 (1,000 rxns/25 μl/rxn)
2 × Multiplex Buffer (High sensitivity)	2 × 1,250 μl	12.50 ml
Multiplex DNA Polymerase (High sensitivity) (10 U/μl)	200 μl	1 ml

Storage

Store at -30 ~ -15℃ and transport ≤0℃.

Applications

Multiplex PCR amplification with DNA as template; detection and typing of pathogens; molecular hybridization detection, etc.

Notes

1. Primer design is critical to the success of multiplex PCR amplification. On the one hand, primer design needs to meet conventional primer design rules to avoid non-specific amplification and failure to amplify. On the other hand, the designed primer pairs are verified by PCR one by one, and then the primer pair with better effect can be selected for multiplex PCR amplification. High-quality primers are recommended.
2. It is recommended that the target fragment does not exceed 3,500 bp.
3. The recommended final concentration of each primer reaction is 0.2 μM. Newly synthesized primers should be calibrated for their true concentrations before use. If the yield of some target fragments is low, the amount of corresponding primers can be appropriately increased to improve the amplification yield.
4. When the amplification efficiency is low, the primer concentration can be appropriately increased; when non-specific amplification occurs, the primer concentration can be appropriately decreased.

Recommended Rules of Primer Design

1. The primer length is 21 - 30 bp, the GC content is 40% - 60%, and the annealing temperature is above 68°C.
2. The overall distribution of primers A, G, C, and T should be as uniform as possible, and regions with high GC or TA content should be avoided. Especially at the 3' end, regions with non-uniform GC content must be avoided.
3. When designing primers, try to avoid the continuous structure of T/C or A/G.
4. The last five bases at the 3' end of the primer cannot contain more than two G or C.

Experiment Process

Reaction System

1. Prepare 5 × Primer Mix: Premix all amplification primers to a final concentration of 1 μM per primer.
2. Prepare the following reaction system in a sterile PCR tube:

2 × Multiplex Buffer[a]	12.5 μl
Multiplex DNA Polymerase (10 U/μl)[b]	1 μl
5 × Primer Mix[c]	5 μl
Template DNA[d]	x μl
ddH_2O	Up to 25 μl

a. It contains dNTP Mix, Mg^{2+}, etc.

b. When non-specific amplification occurs, it can be optimized by reducing the amount of enzyme. Generally speaking, the higher the amount of enzyme, the stronger the amplification ability and the worse the specificity.

c. The recommended final concentration of each primer reaction is 0.2 μM, which can be adjusted between 0.05 - 0.4 μM.

d. The recommended amount of template in a 25 μl reaction system: 50 ng of human genome, 500 pg of plasmid, and 1 - 2.5 μl of cDNA.

PCR Program

Standard Program

Process	Temperature	Time	Cycles
Initial denaturation	95℃	30 sec - 5 min[b]	1
Denaturation	95℃	30 sec	30 - 35[e]
Annealing	60℃[a]	90 sec[c]	
Extension	72℃	60 sec/kb[d]	
Final extension	72℃	10 min	1

a. In most cases, the default annealing temperature will suffice. If the amplification effect is not good, the optimal annealing temperature can be explored through the annealing temperature gradient experiment.

b. The initial denaturation time can be adjusted according to different template types. For the extracted nucleic acid, the initial denaturation time is 30 sec; for direct amplification of whole blood, blood cards, etc., the initial denaturation time can be appropriately extended to 5 min.

c. When amplifying low-copy templates, long fragments or a large number of amplified fragments, the annealing time can be appropriately extended to 3 min to improve the amplification efficiency.

d. The extension time is based on the longest fragment. However, too long extension times can lead to increased non-specific amplification, and amplification specificity can be improved by shortening the extension time.

e. When amplifying trace sample, the amount of amplified product can be increased by increasing the number of cycles. However, excessive cycle numbers may result in increased non-specific amplification.

Fast PCR

Process	Temperature	Time	Cycles
Initial denaturation	95℃	30 sec	1
Denaturation	95℃	15 sec	25 - 30
Annealing	60℃	30 sec[a]	
Extension	72℃	30 sec/kb	
Final extension	72℃	5 min	1

a. The fast program will lose part of the amplification yield. It is recommended to use the standard procedure for the first amplification.

FAQ & Troubleshooting

◇ **No PCR product/low yield.**

① For direct amplification experiments such as blood, blood cards, swabs, etc., confirm that the initial denaturation conditions of the PCR program are 95°C/5 min to fully release the template.

② Use high-quality primers, check whether the primers are degraded, and confirm that the final primer concentration is 0.2 μM.

③ Increase the number of PCR cycles.

④ Reduce the annealing temperature (interval 1 ~ 3℃), and try the annealing temperature gradient if necessary. Confirm that the annealing time is 90 sec, and if necessary, the annealing time can be extended to 3 min.

⑤ Check the amplification performance and specificity of a single pair of primers.

⑥ Use high-quality template; confirm the purity and concentration of DNA template; increase the amount of template used.

⑦ If the product is too long, the primers need to be redesigned.

⑧ Increase the time of extension and final extension.

⑨ Increase the amount of primers used for low-yield or missing amplicon.

◇ **There is non-specific amplification.**

① Decrease the number of cycles.

② Increase the annealing temperature.

③ Reduce the amount of primers used.

④ Redesign primers.

⑤ Reduce the amount of enzymes.

◇ **Blurred bands during electrophoresis.**

① Decrease the number of cycles (reduce 3 cycles each time).

② Reduce the amount of initial template.

③ Increase the time of the final extension to 15 - 30 min.

④ Reduce the electrophoresis voltage; replace with a new electrophoresis buffer.