

Taq Pro Multiplex DNA Polymerase (High specificity)

PM202

Version 22.2



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 specificity version has been carefully optimized for multiplex PCR with superior high amplification specificity. With the optimal buffer system for multiplex PCR, it is compatible with a wide range of product GC content and primer T_m values, and most multiplex PCR assays can be completed at a universal annealing temperature of 60°C.

This product has **excellent amplification efficiency**, and all primers in the reaction system can be effectively used to amplify; it has **broad compatibility** and can amplify primers and templates with different GC contents; it has **superior amplification specificity** and can effectively reduce primer mismatches that caused by nonspecific amplification.

Components

Components	PM202-01 (200 rxns/25 µl/rxn)	PM202-02 (1,000 rxns/25 µl/rxn)
2 × Multiplex Buffer (High specificity)	2 × 1,250 µl	12.50 ml
Multiplex DNA Polymerase (High specificity) (10 U/µl)	200 µl	1 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

Multiplex PCR amplification; pathogen detection and typing; molecular hybridization detection, etc.

Notes

For research use only. Not for use in diagnostic procedures.

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 nonspecific amplification and failure to amplify. On the other hand, the designed primer pairs are verified by PCR one by one, and then the primer pairs 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 3,000 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 nonspecific amplification occurs, the primer concentration can be appropriately decreased.

Primer Design Guidance

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 A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents. Especially at the 3' end, regions with non-uniform GC content must be avoided.
3. 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

1. Reaction System

Prepare 5 × Primer Mix: Premix all amplification primers to a final concentration of 1 µM per primer.

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 ₂ O	Up to 25 µl



- a. It contains dNTP Mix, Mg²⁺, etc.
- b. When nonspecific amplification occurs, it can be optimized by reducing the amount of enzyme. In general, a higher amount of enzyme will lead to stronger amplification ability, resulting in poorer 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.

2. PCR Program

Standard Program

Process	Temperature	Time	Cycles
Initial Denaturation	95°C	30 sec - 5 min ^b	1
Denaturation	95°C	30 sec	} 30 - 35 ^e
Annealing	60°C ^a	90 sec ^c	
Extension	72°C	60 sec/kb ^d	
Final Extension	72°C	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 nonspecific amplification, and amplification specificity can be improved by shortening the extension time.
- e. When amplifying trace sample, the yield of amplification products can be improved by increasing the number of cycles. However, excessive cycle numbers may result in increased nonspecific amplification, and amplification specificity can be improved by reducing the number of amplification cycles.

Fast Program

Process	Temperature	Time	Cycles
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	15 sec	} 25 - 30
Annealing	60°C ^a	30 sec ^c	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	1

- a. The amplification yield decreases under fast program. It is recommended to use the standard procedure for the first amplification.

FAQ & Troubleshooting

◇ No amplification products or 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°C), 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.

◇ Nonspecific amplification

- ① Reduce the number of cycles.
- ② Increase the annealing temperature.
- ③ Reduce the usage of primers.
- ④ Redesign primers.
- ⑤ Reduce the amount of enzymes.

◇ Smear bands

- ① Reduce the number of cycles (reduce 3 cycles each time).
- ② Reduce the amount of initial templates.
- ③ Increase the time of the final extension to 15 - 30 min.
- ④ Reduce the electrophoresis voltage; replace with a new electrophoresis buffer.

