

5×AMPQX® PCR Master Mix(for multiplex PCR)

Cat. no. LM201012-2

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Product Information

Product	5× AMPQX® PCR Master Mix(for multiplex PCR)
Catalog No.	LM201012-2
Size	1 mL/500mL
Storage conditions	This product should be stored at - 20 °C for 2 years.

2.Product description

5 × AMPQX® PCR Master Mix (for Multiplex PCR) is developed based on a new generation of high-fidelity DNA polymerase Canace, and is specifically optimized for multiplex PCR library construction applications in the field of high-throughput sequencing. The Canace enzyme is based on Pyrococcus furiosus DNA Polymerase and is modified by genetic engineering. The protein structure contains the 5' 3' polymerase domain and the modified 3' 5' exonuclease domain. This combination greatly improves the heat resistance and fidelity of the enzyme. It is 98 °C, 52 times more fidelity than Taq DNA polymerase and 6 times more fidelity than common Pfu DNA polymerase. Unique elongation factors were added to the enzyme solution and the amplification was up to 15 sec / kb. This product is a 5 mix PCR mixture, with a large volume range of added templates and primers, making your experiment more flexible (our company has another 2 mix available).

3.Instructions

3.1.Recommended PCR reaction systems.

Components	Volume (μL)	Final concentration
5× AMPQX® PCR Master Mix (for multiplex PCR)	10	-
Template	x	-
Primer mix (X μM)	x	-
ddH ₂ O	Up to 50	-

[Note]:

a) Please mix each components before use.

b) Primers and template: The first round of multiplex PCR for target enrichment, in which the primers and template conditions need to be explored. The concentration of the second round of multiplex PCR is recommended: 10 μ M primer plus about 2 μ L.

3.2.Reaction program

3.2.1.First-round PCR amplification procedure:

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	3min	1
Denaturation	98°C	20 sec	15-45
Annealing	60°C	4-10 min	
Extension	60°C	4-10 min	
Final extension	72°C	5 min	1

[Note]:

- Set the reaction procedure in the above table on the PCR instrument to perform the multiple PCR reaction (set the hot cover temperature to about 105°C).
- Annealing / extension time: 100 heavy PCR recommended 4 min; 1000 heavy recommended 8-10 min according to the primer situation of exploration.

3.2.2 The PCR products were purified by magnetic beads

It is recommended to add 45 μ L magnetic beads to the 50 μ L reaction system for product purification

3.2.3 Second-round PCR amplification procedure:

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	15 sec	25-35
Annealing	60°C	30 sec	
Extension	72°C	1min	
Final extension	72°C	5 min	1

[Note]:Annealing temperature and time: 60°C is recommended, and a temperature gradient can also be set up to explore the optimal temperature of primer annealing as needed. The recommended annealing time is set to 30 sec, which can be adjusted within 10-30 sec. Too long an annealing time may cause the dispersion of the amplification product on the glue.

3.2.4 The PCR products were purified by magnetic beads

It is recommended to add 45 μ L magnetic beads to the 50 μ L reaction system for product purification

4. Notes

- For your safety and health, please wear a lab coat and disposable gloves during operation.
- This product is for research use only!
- It is worth noting that the multiple PCR library building experiment itself is difficult, which usually needs to be optimized. If you encounter difficulties when using this product, you can contact our technical support. We have multiple optimization schemes, which can help you to better build the experimental system