2× Taq PCR Aurora Mix

REF: EG25104-M/L

Storage Condition

-20℃

Components

Component	EG25104M	EG25104L
2× Taq PCR Aurora Mix	5×1 ml	100×1 ml

Description

Taq PCR Aurora Mix is a ready-to-use PCR mix with the concentration of 2×. It offers convenience and reduces the risk of contamination. PCR products synthesized with this mix have an overhang of A bases at the 3' end, enabling direct T/A cloning after purification.

The Mix contains Taq DNA polymerase and a protein with 3' → 5' exonuclease activity, enabling efficient amplification of DNA fragments up to 7 kb with high yields. Coupled with the optimized reaction buffer, it can achieve efficient amplification for different GC contents (ranging from 30% to 70%). In addition, compared with WT-Taq DNA polymerase, the extension rate of Taq PCR Aurora Mix is enhanced by 2 to 4 times, which can effectively shorten the reaction time.

This PCR Mix contains two dyes. The PCR products can be directly loaded for electrophoresis without adding a Loading Buffer, and two indicator bands in blue and red colors will appear during the electrophoresis process. These dyes do not interfere with the efficiency of PCR amplification. However, for experiments that require optical analyses such as absorbance and fluorescence of the PCR products, it is recommended to purify the PCR products prior to analysis.

Quality Control Assays

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 25 μl of Taq PCR Aurora Mix incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 50 μl reaction containing 15 ng of dsDNA fragments and 25 μl of Taq PCR Aurora Mix incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

1. PCR Reaction System

Reagent	Amount	Final Concentration
2× Taq PCR Aurora Mix[a]	25 μl	1×
Forward Primer (10 μM)[b]	1~2 μl	0.2~0.4 μM
Reverse Primer (10 μM)[b]	1~2 μl	0.2~0.4 μM
Template DNA[c]	x μl	
ddH_2O	To 50 μl	

a. Thaw out before use.

b. Recommended final primer concentration is 0.2~0.4 μM. Adjustments can be made in the range of 0.1~1 μM.

c. Optimal reaction concentrations may vary for different templates. For a 50 μl reaction system, the recommended template amounts are approximately 10~200 ng for genomic DNA and 10 pg~5 ng for plasmid or viral DNA.

2. Three-step PCR Program

Step	Temperature	Time	
Initial Denaturation[d]	95℃	3~5 min	
Denaturation	95℃	30 s	← 30~35 Cycles
Annealing[e]	55~65℃	30 s	
Extension[f]	72℃	15~30 s/kb	
Final Extension	72℃	5 min	

3. Two-step PCR Program

Step	Temperature	Time	
Initial Denaturation[d]	95℃	3~5 min	
Denaturation	95℃	30 s	← 30~35 Cycles
Annealing and Extension[e]	60~65℃	30 s/kb	
Final Extension	72℃	5 min	

d. E. coli colony PCR: It is recommended to adjust the pre-denaturation time to 10 min, enabling efficient amplification without pre-lysis. Yeast colony PCR: No need to adjust the pre-denaturation time, but pre-lysis of yeast is required. Recommended lysis methods: Add NaOH to yeast to a final concentration of 20 mM, then pre-lyse at 95℃ for 10 min; or repeatedly freeze-thaw yeast 2~5 times using liquid nitrogen..

e. The annealing temperature should be set according to the Tm value of the primer. If necessary, it is recommended to establish a temperature gradient to identify the optimal temperature for primer-template binding. In addition, the annealing temperature directly determines the amplification specificity. If poor amplification specificity is found, the annealing temperature can be appropriately increased.

f. If the length of the target fragment is less than 3 kb, the extension time can be shortened to 15 s/kb; if the length of the target fragment is more than 3 kb, the recommended extension time is 30 s/kb. To achieve the best amplification effect or a higher yield, it is recommended to use a uniform extension rate of 30 s/kb.

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Notice

1. Primer Design

1. It is recommended that the last base at the 3' end of the primer should be G or C.
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer and avoid hairpin structures at the 3' end of the primer.
3. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1℃ and the Tm value should be adjusted to 55 ~65℃ (Primer Premier 5 is recommended to calculate the Tm value).
4. Extra additional primer sequences that are not matched with the template should not be included when calculating the primer Tm value. It is recommended that the GC content of the primer to be 40%~60%.
5. 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.
6. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
7. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

2. Product Electrophoresis and Staining

It is recommended to use the soaking staining method for post - electrophoresis staining. The gel staining method may cause the red indicator band to diffuse, which is not conducive to accurate band identification, but it has no effect on the blue indicator band. In a 1% TAE buffer, the migration rate of the blue band is approximately equivalent to that of a 1500 bp fragment, while the migration rate of the red band is approximately equivalent to that of a 100 bp fragment.

FAQ & Troubleshooting

Problem	Possible Reason	Solution
No amplification products or low yield	Primer	Optimize primer design
	Annealing temperature	Set temperature gradient and find the optimal annealing temperature
	Primer concentration	Increase the concentration of primers properly
	Extension time	Increase the extension time to 30 s/kb
	Cycles	Increase the number of cycles to 35~40 cycles
	Template purity	Use templates with high purity
	Input amounts of template	Decrease the amount of crude samples; adjust the amount of other samples according to the recommended amount and increase it properly.
Nonspecific products or smear bands	Primer	Optimize primer design
	Annealing temperature	Try to increase the annealing temperature to 65℃ at 2℃ intervals
	Primer concentration	Decrease the final concentration of primers to 0.2 μM
	Extension time	Shorten the extension time properly, when there are nonspecific bands larger than the target band
	Cycles	Decrease the number of cycles to 25~30 cycles
	Template purity	Use templates with high purity
	Input amounts of template	Adjust the template amount according to the recomm ended amount

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