2×Fast Taq PCR Master Mix (with dye)

Product description

Taq DNA polymerase is a thermostable enzyme with the activity of 5'→3' DNA polymerase and weak 5'→3' exonuclease but has no activity of 3'→5' exonuclease. Using the Taq DNA polymerase, DNA sequence exponential amplification can be performed in a system containing primers, dNTPs, template DNA and appropriate buffer, the product will have a dA overhang at 3' end which can be utilized in TA cloning.

This product is a fast PCR mix product containing mutant Taq DNA Polymerase, dNTP, bromophenol blue, and an optimized buffer system, the mutant Taq enzyme is more resistant to EDTA-treated blood and heparin-treated blood. PCR products can be electrophoresis directly after amplification without the addition of loading buffer. Amplification speeds up to 15 sec/kb are suitable for fast PCR reactions, and amplification speeds can come up to 1 sec/kb within amplicon length ≤1 kb which can save PCR time greatly. It is suitable for PCR amplification ≤5 kb using genome as template and PCR amplification ≤10 kb using plasmid and λDNA as template. The product contains protective agent ingredients, which can ensure stability under freeze-thaw cycles.

If the template has more secondary structure, higher GC content, or longer than 5 kb, it is recommended to optimize more conditions or choose a high-fidelity series such as the 2X HyPerFUsion® High-Fidelity Master Mix (With dye) (Cat. K1030) or 2X Hyperfusion plus master mix (With dye) (Cat. K1117）。

If the subsequent experiment is clone, the product Taq DNA Polymerase kit (Cat. K1036) dye-free version is your more suitable choice. If agarose gel electrophoresis is required for PCR products, no loading buffer is required. We recommend our products SYBR Safe DNA Gel Stain (Cat: A8743) or Hyper Gel Red (Cat: A8746) for electrophoresis.

Components and storage

Components	K1116-1 mL	K1116-5x1 mL	K1116-20x1 mL	K1116-50x1 mL	K1116-100x1 mL
2×Fast Taq PCR Master Mix (with dye)	1 mL	5 mL	20 mL	50 mL	100 mL
Store the components at -20°C for 12-24 months.					

Experimental manipulation

1. Set up the PCR reaction system.

COMPONENT	20 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
ddH2O	add to 20 µL	add to 50 µL	
10 µM Forward Primer	0.8 µL	2 µL	0.4 µM
10 µM Reverse Primer	0.8 µL	2 µL	0.4 µM
Template DNA	variable	variable	1-500 ng
2×Fast Taq PCR Master Mix (with dye)	10 µL	25 µL	

Please note:

A. Templates can be cDNA (1-5 µL/50 µL*), animal and plant gDNA* (0.1-1 µg/50 µL), *E. coli gDNA (10-100 ng/50 µL), plasmid D NA (0.1-10 ng/50 µL) or λDNA (0.5-10 ng/50 µL).*

Mix the reaction tubes and centrifuge briefly , then transfer to a thermal cycler immediately.

2. PCR cycling conditions

	Temperature	Time	Cycles
Initial denaturation	95℃	3 min	1
Denaturation	95℃	15 sec	30-35 cycles(variable)
Annealing	~55℃(Depending on primer Tm)	15 sec	
Extension	72℃	1 sec (amplicon≤1kb) 15 sec/kb (amplicon＞1kb)	
Final extension	72℃	5 min	1
Hold	4℃	+∞	1

Please note:

A. Annealing temperature setting: 3-5 degrees (the lower of the two Tm) can be subtracted from Tm to the most annealing temperature. The following is a rough calculation

Formula:

When primers are shorter than 20 bp, Tm(℃*) = 4(G+C)+2(A+T).*

*When primers are longer than 20 bp, Tm(°C) = 81.5 + 0.41 * (% of GC) - (675/length).*

You can also use a temperature gradient to determine the annealing temperature.

B. *This predenaturation condition is suitable for most reactions, but for some highly complex genomic DNA or cDNA templates, the predenaturation time can be extended appropriately.*

C. *If higher yields are expected, the extension time can be appropriately extended to 2 to 5 sec with amplicon ≤1 kb ; Amplicon > 1 kb can be extended to 20 - 30 sec/kb*

3. electrophoresis

If agarose gel electrophoresis is required for PCR products, no loading buffer is need. Our products SYBR Safe DNA Gel Stain (Cat: A8743) or Hyper Gel Red (Cat: A8746) is recommended.

4. Common problems and solutions

Classify	No product or low yields	Miscellaneous bands or diffuse bands
Primers	Optimize primer design	Optimize primer design
Annealing temperature	Set the annealing temperature gradient to find the ideal annealing temperature.	Try increasing the annealing temperature, setting gradient temperature up to 65°C at 2°C intervals
Primer concentration	Increase primer concentration appropriately	Reduce the primer concentration to a final concentration of 0.2 μM.
Extended time	Increase the extension time to 30 sec/kb as appropriate	Extended time can be reduced when there are miscellaneous bands larger than the target band.
The number of cycles	Increase the number of cycles to 35-40 cycles	Reduce the number of cycles to 25-30 cycles
Template purity	Use a high-purity template	Use a high-purity template
Template usage	Rough extraction of samples may require a reduced amount of use; The amount of other samples used is increased according to the recommended amount of the reaction system.	The amount of use is adjusted according to the recommended amount of the reaction system.

Note

1. PCR reactions should be performed in an environment free of contamination with impure DNA. It is recommended to use a "clean" dedicated automatic pipette and spray blocking tip. Always keep positive/negative control DNA or other templates separate from other components. The PCR tubes used should be nuclease-free.
2. Commonly used oligonucleotide primers are typically 20-40 nucleotides in length, preferably with a GC content about 40-60%. The final concentration of each primer in the reaction can be 0.05-1 μM, typically 0.1-0.5 μM.
3. Additives such as DMSO or formamide can be used to improve reaction yields for templates that are difficult to amplify, such as GC-rich sequences.
4. For templates that are difficult to amplify, such as GC-rich sequences, a longer initial denaturation prior to PCR cycling, such as 5 min at 95°C, is recommended to allow the template to be completely denaturated. If colony PCR is used, denaturation for 5 min at 95°C is recommended.
5. The annealing time can set between 15-60 seconds. The annealing temperature is often based on the Tm of the primers, typically 45-72°C. The annealing temperature can be optimized by performing temperature gradient PCR, e.g., starting with setting the gradient 5°C below the calculated Tm.
6. This product is for scientific use only.

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