

DESCRIPTION

KAO-201 High Speed High Fidelity PCR Premix 2× TOROBlue® Flash KOD Dye Mix is developed on the basis of a new generation of modified KOD DNA polymerase, which contains two monoclonal antibodies inhibiting 5' → 3' polymerase activity and 3' → 5' exonuclease activity, and has the following typical characteristics:

1. High specificity hot start PCR can be performed.
2. It not only has that characteristic of high fidelity and high successful amplification efficiency, but also has the characteristic of high-speed extension, and realizes high-speed amplification with the fast speed of 1sec/kb.
3. Has high inhibition resistance, and has excellent amplification performance on crude samples, on template containing inosine or uracil and on template with high GC content.
4. Contains electrophoresis sample loading indicator, can carry out amplification only by adding a template and a primer, and can directly carry out electrophoresis by spotting after the PCR reaction is finished.

COMPONENTS

Item No	Product name	Specification	Quantity
KAO-201	2×TOROBlue® Flash KOD Dye Mix	1mL/tube	5 tubes

APPLICATION

Suitable for PCR reactions using genomic DNA, cDNA, plasmid DNA, DNA containing inosine or uracil, and crude samples as templates. It can also amplify DNA template treated with bisulfite. This product can also be used for metagenomic related experiments.

NOTE

1. Primers are preferably designed at 22-35 bp, and the annealing temperature should be above 60 °C. For long fragment amplification, primers above 25bp are preferred, and the annealing temperature is recommended to be above 65 °C.
2. For high GC content or complex samples, try to add a pre-denaturation step (94 °C, 2 min) before cycling.

PROTOCOL

1. This premix should be fully thawed before use. Gently vortexed and centrifuged at 6000 rpm for 10 seconds.
2. Prepare the following reaction mixture:

Component	Addition amount	Final concentration
2×TOROBlue® Flash KOD DyeMix	25μL	1×
10 μM upstream primer	1.5μL	0.3μM
10 μM downstream primer	1.5μL	0.3μM
DNA template	≧ 5μL	10-200 ng of genomic DNA; Plasmid 1-50 ng; cDNA~750 ng; Live sample or crude extract 5 μL
ddH ₂ O	Make up to 50 μL	

*1. After the components are added, please mix them evenly before placing them in the PCR instrument.

*2. The primer concentration of 0.3 μM (final concentration) is recommended, but when amplifying long fragments of more than 10 kb, the primer concentration of 0.15 μM (final concentration) can improve the amplification product. In addition, when the detection sensitivity is poor, it is suggested that increasing the primer concentration to 0.5 μM (final concentration) may improve it.

CYCLING CONDITIONS

When the T_m value of the primer minus 5°C is lower than 68 °C, it is recommended to use the three-step cycling condition:

Steps	Temperature	Time	Number of cycles
Denaturalization	98°C	10sec	25-45
Annealing	(T _m -5)°C	5sec	
Extend	68°C	1~10sec /kb	

When the T_m value of the primer minus 5°C is higher than 68°C, a two-step cycling condition can be used:

Steps	Temperature	Time	Number of cycles
Denaturalization	98°C	10sec	25-45
Anneal/extension	68°C	5~10 sec/kb	

STORAGE

This reagent should be kept at -20°C. It can be stored at 2 ~ 8 °C for one month, and its quality is not affected by repeated freezing and thawing for not more than 20 times.

Q&A

1. How to increase the amount of amplification products?

Answer: The amount of amplification products can be increased by increasing the number of cycles, setting the optimal annealing temperature, and increasing the amount of template and primer.

2. There are many mixed bands in the electrophoresis of amplification products. How to improve it?

Answer: Mixed bands can be reduced by using two-step conditions, reducing the amount of primer added, increasing the annealing temperature, or optimizing the primer.

RELATED PRODUCTS

Gel Red nucleic acid dye

Item No	Name	Specification	Manufacturer
RSD-100	GelRed Nucleic Acid Staining Dye (10000×)	500 μL/tube	TOROIVD

LED Green Nucleic Acid Dye

Item No	Name	Specification	Manufacturer
GSD-100	LEDGreen DNA Staining Dye (10000×)	500 μL/tube	TOROIVD

Agarose powder

Item No	Name	Specification	Manufacturer
AGR-100	Agarose	100g/bottle	TOROIVD

Seamless cloning kit

Item No	Name	Specification	Manufacturer
FCM-050	TOROIVD® One Step Fusion Cloning Mix	250μL/tube	TOROIVD