

Hieff™ Multiplex PCR Master Mix

Product description

This product is suitable for multiple PCR experiments by two rounds of PCR amplification, and it is suitable for co-detection of DNA pathogenic microorganisms. This kit is based on super multiplex PCR, combined with hot start modification technology and matched with the optimal buffer system, which can realize the re-amplification of Panel with panel multiplicity of 1-2,000. This product is compatible with reverse transcription system, and the GC content of amplicon can cover the range of 25-75%. It has the characteristics of high uniformity, high specificity and high sensitivity. This reagent is suitable for multiple amplification of fragments with different lengths, such as 100-500 bp, and can also be used for the capture of amplicon more than thousand.

Specifications

Cat.No.	12948ES24	12948ES48	12948ES97
Size	24 T	48 T	96 T

Components

Components No.	Name	12948ES24	12948ES48	12948ES96
12948	4× Multiplex PCR Mix	180 µL	360 µL	720 µL

Storage

This product should be stored at -25~-15°C for 12 months.

Note

1. Please operate with lab coats and disposable gloves, for your safety.
2. This product is for research use only.

Instructions

1. Step 1.1st PCR Amplification

A. PCR Reaction system

Components No.	Components	Volume (µL)	stock concentration
12948	4× Multiplex PCR Mix	7.5	1×
-	Primer mix	x	0.1 µM-0.5 µM
-	DNA or cDNA	-	1 ng -400 ng
-	ddH ₂ O	up to 30	-

[Note] : 1) The DNA amount and primer concentration in the above table are recommended, and the optimum concentration can be adjusted according to the specific experimental conditions.

2) Reference suggestion: The concentration of each primer can be set between 0.1 µM and 0.5 µM.

3) The pre-mixed solution already contains enzymes, dNTP, salt ions, etc. needed for amplification, and no additional addition is needed.

B. PCR Reaction program

Step	Temperature (°C)	Time	Number of cycles
Heat lid	105°C	On	
Predenaturation	95°C	3 min	1
Denaturation	95°C	30 sec	X*
Annealing extension	60°C	30 sec-1 min	
Extend	72°C	1 min	
Final extension	72°C	3 min	1
Hold	4°C	-	1

【Note】 : * Lower template input can increase the number of cycles

C. Purification of PCR products (0.9×)

- 1). Preparation: Take out the Hieff NGS™ DNA Selection Beads from the 4°C and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.
- 2). Vortex or fully invert the magnetic beads.
- 3). Add 27 μL Hieff NGS™ DNA Selection Beads (0.9×, Beads:DNA=0.9:1) to PCR product and mix well on a vortex mixer or by pipetting up and down at least 10 times, and incubate at room temperature for 5 min.
- 4). Spin the PCR tube briefly and place it on a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully discard the supernatant.
- 5). Keep the PCR tube on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the tube, incubate at room temperature for 30 sec, carefully remove the supernatant.
- 6). Repeat step 5 once for a total of 2 washing steps.
- 7). Keep the PCR tube on the magnetic stand at all times, completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic stand with the lid open (no more than 5 minutes).
- 8). Remove the PCR tube from the magnetic stand, Elute DNA target from the beads with 12 μL ddH₂O. Mix well on a vortex mixer or by pipetting up and down, and incubate for 5 minutes at room temperature. Briefly spin the tube, and put the tube back on the magnetic stand until the solution is clear, carefully transfer 10 μL of supernatant to a new PCR tube for next step of analysis.

2. 2nd PCR Amplification

A. PCR Reaction system

Components No.	Components	Volume (μL)	stock concentration
12948	4× Multiplex PCR Mix	7.5	1×
	Primer Index mix	6 pmol	-
	PCR products or DNA	10	-
	ddH ₂ O	x	-
	Total	up to 30	

B. PCR Reaction system

Step	Components No.	Components	Volume (μL)
Heat lid	105°C	On	
Predenaturation	95°C	3 min	1
Denatutation	95°C	30 sec	x
Annealing extension	60°C	30 sec	
Extend	72°C	1 min	
Final extension	72°C	3 min	1
Hold	4°C	-	1

【Note】 :

1) Please adjust the annealing time according to the number of multiple amplification primers, and the annealing time can be increased when the number of Panel primers is large.

2) For samples with low template content, the amplification output can be improved by increasing the number of cycles.

C. The number of amplification cycles can refer to the input of the template (the first amplification product), and the reference conditions are as follows.

Amount of DNA template(RT-PCR product)	20 ng	50 ng	100 ng	200 ng
cycle	8 cycles	7 cycles	6 cycles	5 cycles

【Note】 :The above table is the recommendation of the first and second cycle numbers based on 10 ng DNA; If the amount of DNA input is greater than 10 ng, the number of corresponding cycles in the second round can be reduced; When the input is less than 10 ng, the corresponding cycle number of the first and second rounds should be increased accordingly.

D. Post-Amplification Clean-up/Size Selection

1. Preparation: Take out the Hieff NGS™ DNA Selection Beads from the 4°C and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.
2. Vortex or fully invert the magnetic beads.
3. Add 27 μL Hieff NGS™ DNA Selection Beads (0.9×, Beads:DNA=0.9:1) to PCR product and mix well on a vortex mixer or by pipetting up and down at least 10 times, and incubate at room temperature for 5 min.
4. Spin the PCR tube briefly and place it on a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully discard the supernatant.
5. Keep the PCR tube on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the tube, incubate at room temperature for 30 sec, carefully remove the supernatant.
6. Repeat step 5 once for a total of 2 washing steps.
7. Keep the PCR tube on the magnetic stand at all times, completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic stand with the lid open (no more than 5 minutes).
8. Remove the PCR tube from the magnetic stand, Elute DNA target from the beads with 30 μL ddH₂O. Mix well on a vortex mixer or by pipetting up and down, and incubate for 5 minutes at room temperature. Briefly spin the tube, and put the tube back on the magnetic stand until the solution is clear, carefully transfer 25 μL of supernatant to a new PCR tube for next step of analysis.