

## 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× Multiple 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 1.1<sup>st</sup> PCR Amplification

##### A. PCR Reaction system

| Components No. | Components          | Volume (μL) | stock concentration |
|----------------|---------------------|-------------|---------------------|
| 12948          | 4× Multiple PCR Mix | 7.5         | 1×                  |
| -              | Primer mix          | x           | 0.1 μM-0.5 μM       |
| -              | DNA or cDNA         | -           | 1 ng -400 ng        |
| -              | ddH <sub>2</sub> 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                |
| Denatutation        | 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 ddH<sub>2</sub>O. 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. 2<sup>nd</sup> PCR Amplication

### A. PCR Reaction system

| Components No. | Components          | Volume (μL) | stock concentration |
|----------------|---------------------|-------------|---------------------|
| 12948          | 4× Multiple PCR Mix | 7.5         | 1×                  |
|                | Primer Index mix    | 6 pmol      | -                   |
|                | PCR products or DNA | 10          | -                   |
|                | ddH <sub>2</sub> 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 ddH<sub>2</sub>O. 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.