

# Phusion™ Flash

## High-Fidelity PCR Master Mix

### Product codes:

F-548S, 100 reactions 20 µl each (1.0 ml)

F-548L, 500 reactions 20 µl each (5 x 1.0 ml)

Stable for six months from the packaging date. Store at -20°C.

## 1. Introduction

Finnzymes' Phusion™ Flash High-Fidelity PCR Master Mix is a 2x master mix based on modified Phusion Hot Start DNA Polymerase. The unique composition of Phusion Flash PCR Master Mix enables the use of extremely short PCR protocols (15 s/1 kb) with both low and high complexity DNA templates. Phusion Flash PCR Master Mix contains all reagents required for PCR except for the DNA template and primers.

Phusion Flash DNA Polymerase is a proofreading polymerase that contains a unique processivity-enhancing domain, making this polymerase accurate and rapid. Phusion Flash DNA Polymerase is a hot start polymerase utilizing a reversibly binding Affibody® protein.<sup>1,2</sup> This protein inhibits DNA polymerase activity at ambient temperatures, thus preventing amplification of non-specific products. In addition, the Affibody protein blocks the 3'→5' exonuclease activity of the polymerase, preventing degradation of primers and template DNA during reaction setup. At polymerization temperatures, the Affibody protein dissociates from the polymerase rendering the enzyme fully active.

Phusion Flash DNA Polymerase possesses 5'→3' DNA polymerase activity and 3'→5' exonuclease activity. The error rate using Phusion Flash PCR Master Mix is  $9.5 \times 10^{-7}$  when determined with a modified lacI-based method.<sup>3</sup> The error rate is approximately 25-fold lower than that of *Thermus aquaticus* DNA polymerase and 3-fold lower than that of *Pyrococcus furiosus* DNA polymerase. The polymerase is capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb lambda DNA used in Finnzymes' quality control assays.

### Important notes

- Use 98°C for denaturation. (See 4.1 & 4.2)
- Use 2-step protocol or anneal at  $T_m+3^\circ\text{C}$ . (See 3.2 & 4.3)
- Use 15 s/kb for extension. (See 4.4)
- Note: Phusion Flash DNA Polymerase produces blunt end DNA products.

## 2. Guidelines for using Phusion™ Flash PCR Master Mix

Carefully mix and spin down the Phusion Flash PCR Master Mix tube before opening to improve recovery. The PCR setup can be performed at room temperature.

Due to the nature of Phusion Flash DNA Polymerase, optimal reaction conditions may differ from other amplification protocols. Please pay special attention to the conditions listed below when running your reactions. Following the guidelines will ensure optimal enzyme performance.

Table 1. Pipetting instructions.

| Component                        | 20 µl reaction | 50 µl reaction | Final conc. |
|----------------------------------|----------------|----------------|-------------|
| H <sub>2</sub> O                 | add to 20 µl   | add to 50 µl   |             |
| 2x Phusion™ Flash PCR Master Mix | 10 µl          | 25 µl          | 1x          |
| primer A (see 3.2)               | x µl           | x µl           | 0.5 µM      |
| primer B (see 3.2)               | x µl           | x µl           | 0.5 µM      |
| template DNA (see 3.3)           | x µl           | x µl           |             |

Table 2. Cycling instructions.

| Cycle step             | 2-step protocol |               | 3-step protocol |               | Cycles |
|------------------------|-----------------|---------------|-----------------|---------------|--------|
|                        | Temp.           | Time          | Temp.           | Time          |        |
| Initial denaturation   | 98°C            | 10 s          | 98°C            | 10 s          | 1      |
| Denaturation (see 4.2) | 98°C            | 0 or 1 s      | 98°C            | 0 or 1 s      | 30     |
| Annealing (see 4.3)    | -               | -             | X°C             | 5 s           |        |
| Extension (see 4.4)    | 72°C            | 15 s/1 kb     | 72°C            | 15 s/1 kb     |        |
| Final extension        | 72°C<br>4°C     | 1 min<br>hold | 72°C<br>4°C     | 1 min<br>hold | 1      |

## 3. Notes about reaction components

### 3.1 Phusion™ Flash High-Fidelity PCR Master Mix

Phusion Flash PCR Master Mix contains all necessary reaction components for PCR except for template DNA and primers. The composition of the Phusion Flash PCR Master Mix is designed to give optimal results.

When cloning fragments amplified with Phusion Flash DNA Polymerase, blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with DyNAzyme™ II DNA Polymerase (F-501), for example. However, before adding the overhangs it is very important to remove all Phusion Flash DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion Flash DNA Polymerase will degrade the A overhangs, creating blunt ends again. A detailed protocol for TA cloning of PCR fragments amplified with any of the Phusion DNA Polymerases can be found on Finnzymes' website ([www.finnzymes.com](http://www.finnzymes.com)).

### 3.2 Primers

The recommendation for final primer concentration is 0.5 µM. If required, the primer concentration may be optimized between 0.2-1.0 µM.

To shorten the time required for a PCR protocol, it is advisable to design primers suitable for a two-step PCR protocol, if possible. In a two-step PCR protocol, primer annealing and extension occur at 72°C and a separate annealing step can be omitted. However, Phusion Flash PCR Master Mix can also be used when performing a PCR protocol with a separate annealing step (see section 4.3).

When designing primers, the  $T_m$  values should be calculated with the nearest-neighbor method<sup>4</sup> because results from primer  $T_m$  calculations can vary significantly depending on the method used. Instructions for  $T_m$  calculation and a link to a calculator using the nearest-neighbor method can be found on Finnzymes' website ([www.finnzymes.com](http://www.finnzymes.com)).

### 3.3 Template

General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 20 µl reaction volume, or 2.5 pg–25 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 10–100 ng per 20 µl reaction volume, or 25–250 ng per 50 µl reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10 % of the final PCR reaction volume.

## 4. Notes about cycling conditions

### 4.1 Initial denaturation

Denaturation should be performed at 98°C. Due to the high thermostability of Phusion Flash DNA Polymerase, even higher temperatures may be used. Initial denaturation of 10 seconds is recommended for all templates when using Phusion Flash PCR Master Mix.

### 4.2 Denaturation

A very short denaturation step is recommended. For this step, it is usually sufficient that the reaction mixture reaches the required 98°C. If the PCR instrument used does not accept 0 seconds as a value, then a 1-second value can be programmed.

### 4.3 Primer annealing

For minimizing the total PCR cycling time, a two-step PCR protocol is recommended. It is applicable with primers whose  $T_m$  values are, when calculated with Finnzymes'  $T_m$  calculator, at least 69°C or 72°C (primers > 20 nt or ≤ 20 nt, respectively).

Basic rules for primer annealing are: For primers >20 nt, anneal for 5 seconds at a  $T_m$  +3°C of the lower  $T_m$  primer. For primers ≤ 20nt, use an annealing temperature equal to the  $T_m$  of the lower  $T_m$  primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR). **Note:** Do not perform annealing below 50°C.

### 4.4 Extension

The extension should be performed at 72°C. Extension time of 15 seconds per 1 kb is suitable for most templates. Some amplicons can be successfully amplified with even shorter extension times, e.g. 1-5 seconds per 1 kb.

## 5. Troubleshooting

| No product at all or low yield                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>Repeat and make sure that there are no pipetting errors.</li><li>Make sure that the cycling protocol was performed as recommended.</li><li>Optimize annealing temperature.</li><li>Titrate template amount.</li><li>Template DNA may be damaged. Use carefully purified template.</li><li>Increase the number of cycles.</li><li>Check the purity and concentration of the primers.</li><li>Check primer design.</li><li>Increase extension time.</li><li>Increase denaturation time up to 5 seconds.</li></ul> |
| Non-specific products - High molecular weight smears                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <ul style="list-style-type: none"><li>Make sure that the extension time used was not too long. (Recommended extension time is 15 s/kb.)</li><li>Increase annealing temperature or perform a temperature gradient PCR.</li><li>Titrate template amount.</li><li>Reduce the total number of cycles.</li><li>Decrease primer concentration.</li></ul>                                                                                                                                                                                                    |
| Non-specific products - Low molecular weight discrete bands                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <ul style="list-style-type: none"><li>Increase annealing temperature.</li><li>Titrate template amount.</li><li>Shorten extension time.</li><li>Perform a temperature gradient PCR.</li><li>Decrease primer concentration.</li><li>Design new primers.</li></ul>                                                                                                                                                                                                                                                                                       |

## 6. Component specifications

### 6.1 Phusion™ Flash DNA Polymerase

Thermostable *E. coli* DNA Polymerases are purified from recombinant *E. coli* strains. Phusion DNA Polymerases possess the following activities: 5'→3' DNA polymerase activity and 3'→5' exonuclease activity. The Affibody ligand is purified from an *E. coli* strain carrying a plasmid encoding Affibody protein. Phusion Flash DNA Polymerase is free of contaminating endo- and exonucleases.

**DNA amplification assay:** Performance in PCR is tested by the amplification of 7.5 kb genomic DNA and 20 kb lambda DNA.

## 7. References

- Nord *et al.* (1997) *Nature Biotechnol.* 15, 772-777.
- Wikman *et al.* (2004) *Protein Eng., Des. Sel.* 17, 455-562.
- Frey & Suppmann (1995) *Biochemica* 34-35.
- Breslauer *et al.* (1986) *PNAS* 83: 3746-3750.

### Shipping and storage

Phusion Flash PCR Master Mix is shipped on gel ice. Upon arrival, store the components at -20°C. Phusion Flash PCR Master Mix is stable for six months from the packaging date when stored and handled properly.

### Warranty

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