FINNZYMES

Phusion™ Flash

High-Fidelity PCR Master Mix

Product codes:
F-548S, 100 reactions à 20 µl (1.0 ml)
F-548L, 500 reactions à 20 µl (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.[1,2] 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 x 10^{-7} when determined with a modified lacI-based method.[3] 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.

Phusion™ Flash DNA Polymerase is unlike other enzymes. Please read the Quick Guide to modify your protocol for optimal results with Phusion™ Flash PCR Master Mix!

Quick Guide:

- Use 98°C for denaturation. (See 4.1 & 4.2)
- Use 2-step protocol or anneal at Tm+3°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

2.1 Basic reaction conditions for PCR amplifications

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.

Table 1. Pipetting instructions.

Component	20 µl reaction	50 µl reaction	Final conc.
H_2O	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	

2.2 Cycling conditions

Table 2. Cycling instructions.

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

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 Tm values should be calculated with the nearest-neighbor method[4] because results from primer Tm calculations can vary significantly depending on the method used. Instructions for Tm calculation and a link to a calculator using the nearest-neighbor method can be found on Finnzymes' website (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

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.

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 Tm values are, when calculated with our Tm 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 Tm +3°C of the lower Tm primer. For primers ≤ 20nt, use an annealing temperature equal to the Tm of the lower Tm 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). Two-step cycling without an annealing step is recommended for high Tm primer pairs. **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 by using even shorter extension times, e.g. 1–5 seconds per 1 kb.

7. Troubleshooting

No product at all or low yield
• Repeat and make sure that there are no pipetting errors. • Make sure that the cycling protocol was performed as recommended. • Optimize annealing temperature. • Titrate template amount. • Template DNA may be damaged. Use carefully purified template. • Increase the number of cycles. • Check the purity and concentration of the primers. • Check primer design. • Increase extension time. • Increase denaturation time up to 5 seconds.
Non-specific products - High molecular weight smears
• Make sure that the extension time used was not too long. (Recommended extension time is 15 s/kb.) • Increase annealing temperature or perform a temperature gradient PCR. • Titrate template amount. • Reduce the total number of cycles. • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands
• Increase annealing temperature. • Titrate template amount. • Shorten extension time. • Perform a temperature gradient PCR. • Decrease primer concentration. • Design new primers.

8. Component Specifications

8.1 Phusion™ Flash DNA Polymerase

Thermostable Phusion 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.

9. References

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

Storage and shipping

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

Finnzymes Oy warrants that its products will meet the specifications stated on the technical data section of the data sheets, and Finnzymes Oy agrees to replace the products free of charge if the products do not conform to the specifications. Notice for replacement must be given within 60 days of receipt. In consideration of the above commitments by Finnzymes Oy, the buyer agrees to and accepts the following conditions:

- That this warranty is in lieu of all other warranties, express or implied;
- That ALL WARRANTIES OF MERCHANT ABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE ARE HEREBY EXCLUDED AND WAIVED;
- That the buyer's sole remedy shall be to obtain replacement of the product free of charge from Finnzymes Oy; and
- That this remedy is in lieu of all other remedies or claims for damages, consequential or otherwise, which the buyer may have against Finnzymes Oy.

Exclusive terms of sale. Finnzymes Oy does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of Finnzymes Oy. Prices are subject to change without notice.

Recommended guidelines for safe use of the products. Finnzymes Oy recommends that the buyer and other persons using the products follow the N.I.H. guidelines published in the Federal Register, Volume 41, No. 131, July 7, 1976, and any amendments thereto. Finnzymes Oy disclaims any and all responsibility for any injury or damage which may be caused by the failure of the buyer or any other person to follow said guidelines.

Research use only. Since these products are intended for research purposes by qualified persons, the Environmental Protection Agency does not require us to supply Premanufacturing Notice.

Notice to user. The information presented here is accurate and reliable to the best of our knowledge and belief, but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary.

Phusion™ DNA Polymerases Notice to Purchaser: Limited license. The purchase price of this product includes a limited, non-transferable license under U.S. and foreign patents owned by New England Biolabs, Inc. to use this product. No other license under these patents is conveyed expressly or by implication to the purchaser by the purchase of this product. The purchase price of this product includes a limited, non-transferable license under U.S. and foreign patents owned by BIO-RAD Laboratories, Inc., to use this product. No other license under these patents is conveyed expressly or by implication to the purchaser by the purchase of this product.

The quality system of Finnzymes Oy is certified according to standard SFS-EN ISO9001:2000.

Version 1.0, June 2007

FINNZYMES
TOOLS FOR MOLECULAR BIOLOGY

FINNZYMES OY
Keilaranta 16 A, 02150 Espoo, Finland
Tel. +358 9 584 121
Fax +358 9 5841 2200
fz@finnzymes.fi, www.finnzymes.com