thermo scientific

PRODUCT INFORMATION

Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix

Pub. No. MAN0012774
Rev. Date 27 June 2018 (Rev. B.00)

#__

Lot __ Expiry Date __

Store at -20°C

Ordering information

Component	#F-548S 100 rxns	#F-548L 500 rxns
2X Phusion Flash Master Mix	1 mL	5 ×1 mL

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

1. Introduction

Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix is a 2X master mix based on modified Phusion Hot Start II DNA Polymerase. The unique composition of Phusion Flash High-Fidelity 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 the reagents required for PCR except for the DNA template and primers.

Phusion Flash II DNA Polymerase is a proofreading polymerase that contains a unique processivity-enhancing domain, making this polymerase accurate and rapid. Phusion Flash II 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 II DNA Polymerase possesses 5'→3' DNA polymerase activity and 3'→5' exonuclease activity. The error rate using Phusion Flash PCR Master Mix is 9.5×10^{-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. Phusion Flash DNA Polymerase is free of contaminating endo- and exonucleases.The polymerase is capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb lambda DNA used in Thermo Scientific's quality control assays.

2. Source

Thermostable Phusion DNA Polymerases are purified from recombinant *E.coli* strains. The Affibody ligand is purified from an *E.coli* strain carrying a plasmid encoding Affibody protein.

3. Important Notes

- Use 98 °C for denaturation (see 6.1 & 6.2).
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Sections 5.2 and 6.3 carefully.
- Use 15 s/kb for extension (see 6.4).
- **Note:** Phusion Flash II DNA Polymerase produces blunt end DNA products.

4. Guidelines for using Phusion Flash PCR Master Mix

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

Due to the nature of Phusion Flash II 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 (add items in this order)

Component	20 µL rxn	50 µL rxn	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 5.2)	X µL	X µL	0.5 µM
Primer B (see 5.2)	X µL	X µL	0.5 µM
Template DNA (see 5.3)	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 6.2) Annealing (see 6.3) Extension (see 6.4)	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

5. Notes about reaction components

5.1 Phusion Flash High-Fidelity PCR Master Mix

Phusion Flash PCR Master Mix contains all the 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 II 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 *Taq* DNA Polymerase, for example. However, before adding the overhangs it is very important to remove all Phusion Flash II DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion Flash II DNA Polymerase will degrade the A overhangs, creating blunt ends again.

5.2 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 6.3). The results from primer Tm calculations can vary significantly depending on the method used. **Always use the Tm calculator and instructions on website: www.thermofisher.com/tmcalculator to determine the Tm values of primers and optimal annealing temperature.**

5.3 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.

(Continued on reverse page)

6. Notes about cycling conditions

6.1 Initial denaturation

Denaturation should be performed at 98 °C. Due to the high thermostability of Phusion Flash II 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.

6.2 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.

6.3 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 (www.thermofisher.com/tmcalculator), at least 69 °C or 72 °C (primers >20 nt or ≤20 nt, respectively).

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).

6.4 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.

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. • 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.

TECHNICAL SUPPORT

EMEA: ts.molbio.eu@thermofisher.com

Americas & APAC: ts.molbio@thermofisher.com

8. References

1. Nord K. *et al.* (1997) *Nature Biotechnol.* 15: 772–777.
2. Wikman M. *et al.* (2004) *Protein Eng. Des.* Sel. 17: 455–462.
3. Frey M. & Suppmann B. (1995) *Biochemica* 2: 34–35.

CERTIFICATE OF ANALYSIS

DNA amplification assay

Performance in PCR is tested by the amplification of a 7.5 kb fragment of genomic DNA and a 20 kb fragment of lambda DNA.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.