thermo scientific

PRODUCT INFORMATION

Thermo Scientific Phusion U Hot Start PCR Master Mix

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Lot __ Expiry Date __

Store at -20 °C

Ordering information

Component	#F-533S 100 rxns	#F-533L 500 rxns
2X Phusion U Hot Start Master Mix	2 x 1.25 mL	10 x 1.25 mL
Water, nuclease-free	2 x 1.25 mL	10 x 1.25 mL

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

1. Introduction

Thermo Scientific™ Phusion™ U Hot Start DNA Polymerase is a modified Phusion DNA Polymerase which efficiently amplifies uracil-containing templates. It can be used to minimize carry-over contamination in conjunction with dUTP and uracil-DNA glycosylase (UDG) and for amplification of bisulfite-treated and damaged DNA. A unique processivity-enhancing domain makes this *Pyrococcus*-like proofreading enzyme extremely processive, accurate and rapid. Phusion U Hot Start DNA Polymerase is capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb λ DNA used in Thermo Fisher Scientific quality control assays. Phusion U Hot Start DNA Polymerase combines the DNA polymerase and a reversibly bound, specific Affibody™ protein[2,3], which inhibits DNA polymerase and 3'→5' exonuclease activities at ambient temperatures, thus preventing the amplification of non-specific products and degradation of primers and template DNA during reaction setup. At polymerization temperatures, the Affibody molecule is released, rendering the polymerase fully active. Phusion U Hot Start DNA Polymerase does not require any separate activation step in the PCR protocol.
Phusion U Hot Start PCR Master Mix is a convenient 2X mix containing Phusion U Hot Start DNA Polymerase, nucleotides and optimized reaction buffer including $MgCl_2$. Only template and primers need to be added by the user.

2. Source

Thermostable Phusion U DNA Polymerase is 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 -30 s/kb for extension (see 6.4).
- **Note:** Phusion U Hot Start DNA Polymerase produces DNA products with blunt ends.

4. Guidelines for using Phusion U Hot Start PCR Master Mix

Carefully mix and spin down the Phusion U Hot Start PCR Master Mix tube before opening to ensure homogeneity and improve recovery. PCR setup can be performed at room temperature.
Due to the novel nature of Phusion U Hot Start 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 U Hot Start 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	X µL	X µL	See section 5.3 for guidelines

Note. For prevention of carryover contamination, use Thermo Scientific™ Uracil-DNA Glycosylase (UDG) and dUTP. Typically, dUTP is added to a final concentration of 0.2 mM. For longer amplicons, a lower dUTP concentration (0.1 mM) may be required for high yields.

Table 2. Cycling instructions

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation (see 6.2)	98°C	5-10 s	98°C	5-10 s	25 - 35
Annealing (see 6.3)	-	-	X°C	10-30 s	
Extension (see 6.4)	72°C	15-30 s/kb	72°C	15-30 s/kb	
Final Extension	72°C 4°C	5 - 10 min hold	72°C 4°C	5 - 10 min hold	1

Note. For carryover contamination prevention with UDG, perform UDG pre-treatment for 2 min at 50°C and 10 min inactivation at 95°C. To avoid PCR product degradadtion by UDG, which gains back its activity when the PCR mix cools below 55°C, store the PCR reaction after cycling at -20°C for long term, or at +4°C for up to 2 days.

5. Notes about reaction components

5.1. Phusion U Hot Start PCR Master Mix

Phusion U Hot Start PCR Master Mix contains all the necessary reaction components for PCR except for template DNA and primers. The composition of Phusion U Hot Start PCR Master Mix is designed to give optimal results. It provides 1.5 mM $MgCl_2$ and 200 µM of each dNTP in final reaction concentration.
When cloning fragments amplified with Phusion U Hot Start 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 (for example with *Taq* DNA polymerase). However, before adding the overhangs it is very important to remove all Phusion U Hot Start DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion U Hot Start DNA Polymerase will degrade the A overhangs, creating blunt ends again.

5.2. Primers

The recommended final primer concentration is 0.5 µM. If required, the primer concentration may be optimized in the range between 0.2 µM and 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 U Hot Start 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.

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6. Notes about cycling conditions

6.1. Initial denaturation

Denaturation should be performed at 98°C. Due to the high thermostability of Phusion U Hot Start DNA Polymerase even higher than 98°C denaturation temperatures can be used. We recommend a 30-second initial denaturation at 98°C for most templates. Some templates may require longer initial denaturation time, and the length of the initial denaturation time can be extended up to 3 minutes.

6.2. Denaturation

Keep the denaturation as short as possible. Usually 5-10 seconds at 98°C is enough for most templates. Note: the denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the cycler.

6.3. Primer annealing

The optimal annealing temperature for Phusion U Hot Start DNA Polymerase may differ significantly from that of *Taq*-based polymerases. **Always use the Tm calculator and instructions on website: www.thermofisher.com/tmcalclator to determine the Tm values of primers and optimal annealing temperature.**

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). A 2-step protocol is recommended when primer Tm values are at least 69°C (> 20 nt) or 72°C (≤ 20 nt) when calculated with Thermo Scientific Tm calculator. In the 2-step protocol the combined annealing/extension step should be performed at 72°C even when the primer Tm is > 72°C.

6.4. Extension

The extension should be performed at 72°C. The extension time depends on the length and complexity of the amplicon. For low complexity DNA (e.g. plasmid, lambda or BAC DNA) use an extension time of 15 seconds per 1 kb. For high complexity genomic DNA, 30 seconds per 1 kb is recommended. For some cDNA templates, the extension time can be increased up to 40 seconds per 1 kb to obtain optimal results.

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 by up to 5 seconds. • If UDG is added, make sure that the temperature during PCR cycling is always higher than 55°C.
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. References

1. Frey M. & Suppmann B. (1995) *Biochemica* 2: 34–35.
2. Nord K. et al. (1997) *Nature Biotechnol*. 15: 772–777.
3. Kinde I.et al. (2011) *Proc Natl Acad Sci USA* 108:9530–9535.

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