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# FAQs for Phusion® High-Fidelity DNA Polymerase

1. **What has changed?**
   See chart for Phusion® products sold by New England Biolabs that are now manufactured by New England Biolabs and have new product numbers.

2. **What are the advantages to using Phusion® High-Fidelity DNA Polymerase?**
   Phusion® High-Fidelity DNA Polymerase's processivity-enhancing domain results in shorter extension times, more robust and high yield amplification, and the ability to extend long templates in a fraction of the time, making Phusion a superior choice for cloning. Phusion is suitable for all PCR applications requiring greater accuracy or long amplicons.

3. **Have the formulations or any other characteristics of these products changed now that they are manufactured by NEB?**
   No. NEB's Phusion products are manufactured at our production facilities in Ipswich, MA to the same specifications as those from Finnzymes/Thermo Scientific. They have been extensively tested and quality controlled, and there are no measurable differences between these products.

4. **Can I continue to use the same reaction conditions if I use Phusion products with the new product numbers?**
   Yes. There is no need to change any parameters of your reactions.

5. **Are the prices and sizes of these products the same now that they are manufactured by NEB?**
   Yes. All prices and product sizes will remain the same.

6. **Are the DNA fragments produced by Phusion® High-Fidelity DNA Polymerase/Phusion® High-Fidelity PCR Master Mix blunt-ended or do they have the single-base 3´ overhang that*Taq* DNA Polymerase yields?**
   Phusion High-Fidelity DNA Polymerase and Master Mixes produce blunt end products.

7. **My template is GC rich or supercoiled. How can I optimize my product yield using Phusion® High-Fidelity DNA Polymerase?**
   * Add DMSO to a final concentration of 2-8%. This often requires a reduction in annealing temperature by 2-5°C.
   * Optimize initial denaturation time. For most templates the optimal initial denaturation time is 30 seconds, but up to 3 minutes can be used for complex templates.
   * If HF buffer has failed, try using GC buffer. GC buffer allows better amplification of GC rich templates, but slightly lowers fidelity.
   Alternatively, try Q5 High-Fidelity DNA Polymerase (NEB #M0491) and include the Q5 High GC Enhancer additive.

**8. What is the error rate of Phusion® High-Fidelity DNA Polymerase?**
The error rate is 4.4 X $10^{-7}$ in Phusion HF buffer and 9.5 X $10^{-7}$ in GC buffer.

**9. I am having trouble amplifying a template that is longer than 5kb. How can I optimize my product yield using Phusion® High-Fidelity DNA Polymerase?**
* Use more template. Sample concentration may be too low.
* Template DNA may be damaged. Use carefully purified template.
* Optimize enzyme concentration by testing a titration of enzyme in the reaction (0.25-2 units/50µl reactions)
* Increase number of cycles.
* If HF buffer has failed, try using GC buffer. GC buffer allows better amplification of GC rich and longer templates, but slightly lowers fidelity.
* Lengthen extension time.

**10. Why do I see no product or low yield on an agarose gel after a PCR using Phusion® High-Fidelity DNA Polymerase?**
* Be sure to use an annealing temperature determined by the nearest neighbor method (or Tm is this subscripted calculator).
* Use fresh high-quality nucleotides. It is important to avoid dNTP mixes that contains dUTP. * Use more template. Sample concentration may be too low.
* Template DNA may be damaged. Use carefully purified template.
* Lengthen extension time.
* Increase cycle number.
* Optimize enzyme concentration.
* Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 98°C or higher.
* The DNA template may be GC rich, or supercoiled: see above.

**11. Why are there high molecular weight smears or DNA in the wells of an agarose gel after a PCR using Phusion® High-Fidelity DNA Polymerase?**
Strong protein binding to the DNA may be preventing proper gel separation. Try adding 1% SDS to the loading dye just before running the gel.
Or non-specific products are being amplified. To avoid this we suggest the following:
* Reduce polymerase concentration.
* Shorten extension time.
* Reduce total number of cycles.
* Increase annealing temperature or try 2-step protocol.
* Optimize $Mg^{2+}$ concentration.
* Lower primer concentration.

**12. Will Phusion® High-Fidelity DNA Polymerase incorporate dUTPs?**
No.

**13. Why are there low molecular weight discrete bands on an agarose gel after a PCR using Phusion® High-Fidelity DNA Polymerase?**
Non-specific products are being amplified. To avoid this we suggest the following:

* Raise the annealing temperature.
* Lower polymerase concentration.
* Shorten extension time.
* Optimize $Mg^{2+}$ concentration.
* Titrate template amount.
* Lower primer concentration.

**14. I'd like to clone a fragment amplified with Phusion® High-Fidelity DNA Polymerase/Phusion® High-Fidelity PCR Master Mix. Do I have to blunt end clone?**

Blunt end cloning is recommended. However, if TA cloning is required, 3´A-overhangs can be added with a different polymerase. It is very important to remove all the Phusion High-Fidelity DNA Polymerase first by purifying the PCR product. The proofreading activity of Phusion is very strong, so any residual polymerase will degrade the A overhangs as they are added. *Taq* DNA Polymerase or Klenow($exo^-$) DNA Polymerase are excellent options for A-overhang addition. We recommend ligating immediately so the 3´A-overhangs will not be lost during storage.

**15. I see foaming when my Phusion® DNA Polymerase amplified PCR products are spotted on microarray slides.**

Use detergent-free reaction buffers. Detergent-free Phusion® HF Buffer (NEB# B0520) or Detergent-free Phusion® GC Buffer (NEB# B0521) are recommended.

**16. What should my primer concentration be when using Phusion® High-Fidelity DNA Polymerase/Phusion® High-Fidelity PCR Master Mix?**

Between 200 nM and 1 µM. 500 nM is recommended.

**17. Is Phusion High-Fidelity DNA Polymerase stable at room temperature?**

Phusion DNA Polymerase retained full activity after ~2 months at room temperature.