SynPhusion Gold Polymerase

High-Fidelity DNA Polymerase with a density reagent and tracking dyes

CAUTION! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from synthbioenzymes.com.

The protocols and storage conditions detailed below ensure that the SynPhusion Gold DNA Polymerase can function at its highest potential. These guidelines cover standard amplification protocols for all PCR reactions and unique specifications tested to provide optimal conditions for SynPhusion Gold DNA Polymerase.

1. Product description

SynPhusion gold contains SynPhusion High-Fidelity DNA Polymerase and 5X SynPhusion Gold Buffer. The buffer has two tracking dyes and a density reagent for directly loading PCR results onto a gel. The density reagent and dyes work well with downstream applications including DNA sequencing, ligation, and restriction digestion without interfering with the SynPhusion DNA polymerases' exceptional performance.

SynPhusion is a high-efficiency DNA Polymerase enzyme that possesses nearly 50x higher fidelity than the traditionally used Taq polymerase. SynPhusion DNA Polymerase also possesses 3´→ 5´ exonuclease activity and generates blunt-ended products.

SynPhusion DNA polymerase is a highly accurate and fast enzyme, therefore making it the perfect option for cloning. With its low error rate, high processivity, and strong proofreading activity it is optimal for even long, difficult and GC rich templates.

The buffer provided with this kit ensures that the PCR reaction takes place at optimal conditions.

2. Contents

SynPhusion Gold package information

P002S	100 U (1 U/µl) Material provided: SynPhusion DNA Polymerase 100 U (100 µl), 5x SynPhusion Gold DNA Polymerase Buffer (2 x 1.5 ml), and 100 mM $MgSO_4$ solution (1.5 ml)
P002M	500 U (1 U/µl) Material provided: SynPhusion DNA Polymerase 500 U (500 µl), 5x SynPhusion Gold DNA Polymerase Buffer (10 x 1.5 ml), and 100 mM $MgSO_4$ solution (2x 1.5 ml)
P002L	2000 U (1 U/ µl) Material provided: SynPhusion DNA Polymerase 2x1000 U (2x1000 µl), 5x SynPhusion Gold DNA Polymerase Buffer (30 x 1.5 ml), and 100 mM $MgSO_4$ solution (5x 1.5 ml)

3. Storage conditions for SynPhusion DNA Polymerase and SynPhusion Gold Buffer

Although our experiments confirm that the SynPhusion DNA Polymerase is viable after incubation at room temperature or 4°C for 2 weeks, we recommend storing all components at -20°C to ensure its quality.

4. Guidelines for using SynPhusion DNA Polymerase

All reaction preparations should be done over ice with sterile lab equipment and should be mixed and centrifuged prior to use. It is suggested to add the SynPhusion DNA Polymerase last in order to the reaction since the enzyme exhibits 3´→5´ exonuclease activity that can degrade primers in the absence of dNTPs. All components of the reaction mix should be mixed and centrifuged gently. SynPhusion Gold is provided with 10x SynPhusion Gold Buffer which ensures that the PCR reaction takes place at optimal conditions and it contains 1.5 mM $MgSO_4$ at final reaction concentrations. Separate tubes of 100 mM $MgSO_4$ solutions are provided for further optimization.

4.1 Standard PCR reaction protocol

Component	20 µl reaction	50 µl reaction
10x SynPhusion Gold DNA Polymerase buffer	2 µl	5 µl
dNTP (10mM)	0.4 µl	1 µl
SynPhusion DNA Polymerase	0.4 µl	1 µl
10 µM Forward Primer	1 µl	2.5 µl
10 µM Reverse Primer	1 µl	2.5 µl
Template DNA	variable	variable
H_2O	to 20 µl	to 50 µl

4.2 Cycling conditions for a routine PCR

Step	Temperature (°C)	Duration
Initial Denaturation	98 °C	1-2 minutes
Denaturation	98 °C	5-15 seconds
Annealing	45-72 °C	10-30 seconds
Extension	72 °C	15-30 seconds per kb
Final Extension	72 °C	5-10 minutes
Hold	4-10 °C	-

4.3 Detailed information about cycling conditions

Initial denaturation Denaturation should be performed at 98°C, SynPhusion DNA Polymerase has high thermal stability. We recommend 1-2 minutes of initial denaturation, but it can be longer for some templates.

Denaturation Denaturation should be 5–15 seconds at 98°C for most templates.

Primer annealing The optimal annealing temperature for SynPhusion DNA Polymerase may differ significantly from Taq–based polymerases. SynPhusion DNA Polymerase tolerates a wide array of annealing temperatures so if there are aspecific products you should probably choose an annealing temperature that is more specific to your primers. For calculating the optimal annealing temperature always use an online calculator specific to phusion polymerases (there is no difference in optimal annealing temperature of our product from other companies). If necessary, use a temperature gradient to find the optimal annealing temperature. For two-step cycling, the gradient can be set as high as the extension temperature. Two-step cycling without an annealing step is recommended for high T_m primer pairs.

Extension The extension should be performed at 72°C. Extension time depends on amplicon length and complexity. Generally, an extension time of 15 seconds per kb can be used. For low complexity 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.

Cycle number Generally, 25–35 cycles is sufficient.

4.4 Information about the reaction components

Enzmye The standard enzyme amount is 1 unit per 50 µl reaction, but optimal amounts could range from 0.5–2 units per 50 µl reaction depending on amplicon length and difficulty. Do not exceed 2 units/50 µl reaction, especially for amplicons longer than 5 kb.

Buffer The SynPhusion Gold Buffer contains 1.5 mM $MgSO_4$ in final reaction conditions.

Primers Ordering primers in different purification can increase the efficiency of the PCR reaction, but experiments show that standard desalted primers will suffice. We recommend making a primer master mix for the reaction, which contains both forward and reverse primers diluted to optimal concentration (10µM).

Template It's very important to use high quality, purified DNA to greatly enhance the success of PCR. Recommended amounts of DNA template is 50-250 ng for genomic and 1 pg-10 ng for low complexity DNA (e.g. plasmid, lambda or BAC DNA) templates for a 50 µl reaction. If the template DNA is not purified from reaction, e.g. a cDNA synthesis reaction mixture is used as a template, the volume of the template should not exceed 10% of the final PCR reaction volume.

Mg^{2+} Mg^{2+} is critical to achieve optimal activity with SynPhusion DNA Polymerase since it is a magnesium dependent enzyme. Therefore, the concentration of Mg^{2+} significantly affects the activity of the enzyme and the PCR reaction. The final Mg^{2+} concentration in SynPhusion Gold Buffer is 1.5 mM. Excessive Mg^{2+} stabilizes the DNA double strand and can prevent complete denaturation of DNA. Excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity. Conversely, inadequate Mg^{2+} may lead to lower product yield. The optimal Mg^{2+} concentration also depends on the dNTP concentration, the specific template DNA and the sample buffer composition. In general, the optimal Mg^{2+} concentration is 0.5 to 1 mM over the total dNTP concentration for standard PCR. If the primers and/or template contain chelators such as EDTA or EGTA, the apparent Mg^{2+} optimum may be shifted to higher concentrations. If further optimization is needed, increase Mg^{2+} concentration in 0.2 mM steps.

dNTP High quality dNTPs should be used. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the use of these analogues or primers containing them is not recommended. For optimal results use 200 µM of each dNTP.

Additives Targets with GC-rich sequences or secondary structure, may be hard to amplify and the amplification may be improved by the presence of additives such as DMSO in a recommended 3% final concentration, but it can be further optimized. For further optimization the concentration of DMSO can be increased in 2% increments. In some cases, DMSO may also be required for supercoiled plasmids to relax for denaturation. It is important to note that a high concentration of DMSO decreases the primer Tm. It has been reported that 10% DMSO decreases the annealing temperature by 5.5–6.0 °C. DMSO is not recommended for amplicons with very low GC % or amplicons that are > 20 kb. SynPhusion Gold DNA polymerase is also compatible with other additives such as formamide, betaine or glycerol.

5. Troubleshooting

Visit our online FAQ for tips and tricks and troubleshooting information:

https://synthbioenzymes.com/product/synphusion-polymerase/