GENAXXON bioscience
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G5 HiFi DNA Polymerase kit

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (DNA-directed); EC 2.7.7.7

	Cat#	M3124.0100	M3124.0500	Colour code of cap
Component				
20X G5 HiFi DNA polymerase S enzyme solution		100µL	500µL	red
20X G5 HiFi DNA polymerase E enzyme solution		100µL	500µL	green
10X buffer with dNTPs		200µL	1000µL	yellow

Description

The Genaxxon bioscience G5 HiFi DNA Polymerase kit consists of our next generation G5 HiFi DNA polymerases S and E, whereas S stands for improved specificity and E stands for improved inhibitor resistance efficiency. Different templates may require a different fine tuning for optimal results. To assist with this need, we have developed our new polymerases in two versions. This allows our customers to tailor the product to their own unique needs by selecting either S or E to work with. Both enzymes are specially designed proofreading polymerases possessing 5'-3' DNA polymerase and 3'-5' proof reading exonuclease activity. Neither enzymes are Sso7D-fusion proteins but are completely newly developed proofreading polymerases. Both enzymes provide extremely high speed and high fidelity with an extremely low error rate (about 280 times more accurate than Taq).

Amplification efficiency is the measurement of fold amplification per PCR cycle and is expressed as a fraction or percentage relative to perfect doubling (a PCR resulting in perfect doubling would exhibit 100 % amplification efficiency). A small change in amplification efficiency is magnified over a multiple-cycle PCR, causing significant changes in product yield. For example, in a 25-cycle PCR, a 5% improvement in amplification efficiency can translate to a two-fold increase in product yield. Lower-efficiency reactions not only produce lower yields but take longer to reach saturation than high-efficiency reactions, increasing the chances of generating primer-dimers and undesirable by-products.

Usage : **Gibson Assembly®, Long Range PCR, Fast PCR, High-Fidelity PCR, HotStart PCR, etc.**

Application:
Amplification of bisulfite-converted, deaminated DNA
FFPE
High-Fidelity PCR
Gibson Assembly.
Long Range PCR.
Fast PCR
Primer extension reactions
PCR cloning and blunt-end amplification product generation

Concentration: 20X enzyme solution in storage buffer

Storage: -20°C

10X Reaction buffer: Complete buffer including dNTPs.

Quality control: Activity, SDS-PAGE purity, absence of endonucleases/nickases

Note: **Recommended elongation time is 20 seconds per 1kb of low complexity target!**

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Reaction Setup

Pipette the following into a PCR tube and mix to create a final volume of 20µL. We recommend dispensing all reagents on ice, adding the enzyme last, since the G5 HiFi DNA Polymerases exhibit 3´→ 5´ exonuclease activity that can degrade primers.

It is important to vortex and mix all buffers before use to remove any gradients that may result from repeated freeze/thaw steps.

Prepare a master mix for the appropriate number of samples to be amplified.

Due to the nature of G5 HiFi DNA Polymerases and the special buffer, the optimal reaction conditions may differ from PCR protocols for standard DNA polymerases and for similar products from competitors. Due to the salt concentration in the reaction buffer, G5 HiFi DNA Polymerases tend to work better at elevated annealing temperatures.

Please do not use your standard pipetting scheme but the one shown below and evaluate the optimal reaction conditions empirically by trying different annealing temperatures, e.g., from -2°C up to +2°C of the already used annealing temperature.

Following the guidelines will ensure optimal enzyme performance.

Table 1: Pipetting scheme

Components	Quantities	Final Concentration
10X G5 Buffer including dNTPs	2µL	<1µg
Primer 1 (10 µM):	1µL	0.1 µM - 0.5 µM
Primer 2 (10 µM):	1µL	0.1 µM - 0.5 µM
20X G5 HiFi DNA-Polymerase	1µL	1X
Template DNA	x µL	
Sterile, bidest. water	up to 20µL	

Transfer the reactions to the thermocycler.

Suggested PCR Protocol

Step	Cycles	Temperature	Time
Initial step	1		
Denaturation		94°C	5 minutes
Amplification	30-40		
Denaturation		94°C	30 seconds
Annealing		Tm -3°C to -4°C***	30 seconds
Extension		72°C	20 sec / kb **
Final			
Extension	1	72°C	5 minutes

* Cycling times and cycling numbers are proposals and must be adjusted to your individual needs.
** The longer the template to be amplified, the longer the amplification time required.
*** Annealing is normally in the range of 3°C to 4°C below Tm.

Analysis of the PCR result

Analyse the amplification reaction by gel electrophoresis using acrylamide or agarose gel of appropriate percentage or process accordingly.

Note: For every template/primer pair the optimal reaction conditions must be evaluated empirically, changing the primer/template ratio, and the cycle parameters (time and temperatures).

Note: The optimal amount of enzyme depends on the amount of template and the length of the PCR product. Usually, 1µLof G5 HiFi DNA Polymerase per 20µL PCR reaction volume gives good results. Nevertheless, the working range can range from 0.5 to 2.0µL per 20µL.

Note: Low complexity DNA (plasmid, lambda or BAC DNA): Use 1 pg – 10 ng per 50 µL PCR reaction. Complex DNA (genomic DNA): use 50 ng – 250 ng per 50 µL PCR reaction.

Note: The optimal annealing temperature for G5 HiFi DNA Polymerases may differ significantly from that of *Taq* based PCR.

Note: The extension should be performed at 72°C. Extension time depends on amplicon length and complexity. For low complexity DNA (e.g., plasmid, lambda or BAC DNA) use an extension time of 20 seconds per 1 kb. **For high complexity genomic DNA 30 seconds per 1 kb is recommended.**

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Trouble shooting:

No PCR product at all or low yield

- Repeat and make sure that there are no pipetting errors.
- Sample concentration may be too low. Use more template.
- Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
- Increase extension time.
- Increase the number of cycles.
- Optimize annealing temperature.
- Optimize enzyme concentration.
- Titrate DMSO (2-8 %) in the reaction.
- Optimize the denaturation time.
- Check the purity and concentration of the primers.
- Check primer design.
- Try using the alternative G5 HiFi DNA Polymerase.

Non-specific products - High molecular weight smears

- Decrease enzyme concentration.
- Decrease extension time.
- Reduce the total number of cycles.
- Increase annealing temperature or try 2-step protocol.
- Vary denaturation temperature.
- Decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

- Increase annealing temperature.
- Decrease extension time.
- Decrease enzyme concentration.
- Titrate template amount.
- Decrease primer concentration.
- Design new primers

Check if annealing temperature has been calculated correctly.
The optimal annealing temperature of primers lies about 5°C below the TM of the oligo.

TM calculation: For oligos up to 15 bases: TM = 4x(G+C) + 2x(A+T)
For larger oligos: TM = 81.5+16.6([log10[J*]) +0.4(%G+C)-(600/n)-0.63(%FA)

A = Adenosine, C = Cytosine, G = Guanine, T = Thymidine, FA = Formamide, J* = concentration of monovalent cations, n = number of bases; TM = calculated melting temperature.

Remark: If primers with mismatches are used the G5 HiFi DNA Polymerases **must** be added immediately before the start of the PCR. Do **not** let the reaction mix stand at room temperature since primers will be degraded by the G5 HiFi DNA Polymerases.