

## SciPhi™ High Fidelity DNA Polymerase (2U/μl)

Catalogue No. NXG344

Catalogue No.	Product Components					
	SciPhi™ Hi-Fi DNA Polymerase (2U/μL)	SciPhi™ 5X Hi-Fi Buffer	SciPhi™ GC Buffer	SciPhi™ MgCl <sub>2</sub> Solution (50 Mm)	SciPhi™ DMSO	SciPhi™ Nuclease Free Water
NXG344-L	100 U (50μl)	1.5 ml x 2	1.5 ml	1.5 ml	500 μl	1.5 ml x 2
NXG344-M	50 U (25μl)	1.5 ml	0.75 ml	0.75 ml	250 μl	1.5 ml
NXG344-S	25 U (12.5μl)	0.75 ml	0.375 ml	0.375 ml	125 μl	0.75 ml
NXG344-XS	10 U (05μl)	0.3 ml	0.15 ml	0.15 ml	50 μl	Not Provided

Both SciPhi™ 5X HF Buffer and 5X GC Buffer provide 1.5 mM MgCl<sub>2</sub> in final reaction conc.  
Store kit and all components at -20°C

### Product Description

SciPhi™ High Fidelity DNA Polymerase is engineered to excel across a broad spectrum of PCR applications. Combining a *Pyrococcus*-like enzyme with a domain that enhances processivity, this polymerase efficiently produces lengthy amplicons with exceptional accuracy and speed, even when challenged by complex templates. Its high fidelity renders it particularly advantageous for cloning purposes. In an assessment utilizing a lacI-based method adapted from prior research, the error rate of Hi-Fi DNA Polymerase in HF Buffer is measured at  $4.4 \times 10^{-7}$ . This rate stands notably superior, approximately 50-fold lower than that of *Taq* DNA polymerase and 6-fold lower than that of *Pyrococcus furiosus* DNA polymerase.

**SciPhi™ Hi-Fi DNA Polymerase**- Optimum enzyme quantity varies based on template quantity and PCR product length. Typically, 1 unit SciPhi™ High-Fidelity DNA Polymerase per 50 μl reaction yields good outcomes. However, the optimal amount can vary between 0.5 and 2 units per 50 μl reaction, depending on the length and complexity of the amplicon. It is advised not to exceed 2 units per 50 μl (0.04 U/μL), particularly for amplicons exceeding 5 kb in length.

**SciPhi™ 5X Hi-Fi and 5X GC Buffer**- The error rate of Hi-Fi DNA Polymerase in HF Buffer ( $4.4 \times 10^{-7}$ ) is lower compared to GC Buffer ( $9.5 \times 10^{-7}$ ). Therefore, HF Buffer is recommended as the primary choice for high-fidelity amplification. However, GC Buffer can enhance the performance of Hi-Fi DNA Polymerase on some complex templates, such as GC-rich sequences or those with intricate secondary structures.

**SciPhi™ MgCl<sub>2</sub> Solution**- The conc. of Mg<sup>2+</sup> is crucial for SciPhi™ High-Fidelity DNA Polymerase due to its dependence on magnesium ions. Excessive Mg<sup>2+</sup> stabilizes the DNA double strand, hindering complete denaturation, and promotes non-specific primer binding, thereby reducing specificity. Conversely, insufficient Mg<sup>2+</sup> can decrease product yield. Typically, for standard PCR, a Mg<sup>2+</sup> conc. of 0.5 to 1 mM higher than the total dNTP concentration is recommended. Templates or primers containing chelators like EDTA or EGTA may require higher Mg<sup>2+</sup> concentrations for optimal performance.

**SciPhi™ DMSO**- The recommended conditions for amplifying GC-rich templates with High-Fidelity DNA Polymerase include using 3% DMSO as a PCR additive, which facilitates the denaturation of templates rich in GC content. For further optimization, the amount of DMSO can be increased incrementally by 2%. In specific cases, such as with supercoiled plasmids, DMSO may be necessary to aid in template relaxation for effective denaturation.

### General Information

**Concentration**- 2U/μL

**SciPhi™ High-Fidelity DNA Polymerase** (thermostable) is purified from an *E.coli* strain expressing the cloned Hi-Fi DNA Pol gene. It

shows 5'→3' DNA polymerase activity and 3'→5' exonuclease activity.

**Storage buffer**- 20 mM Tris-HCl (pH 7.4 at 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 200 μg/mL BSA and 50% glycerol.

**Definition of Activity Unit**- One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into a polynucleotide fraction at 74°C in 30 min.

**SciPhi™ 5X Hi-Fi and 5X GC Buffer**- Both contains 7.5 mM MgCl<sub>2</sub>, which provides 1.5 mM MgCl<sub>2</sub> in final reaction conditions.

**SciPhi™ 50Mm MgCl<sub>2</sub> solution**- Both Buffers supply 1.5 mM MgCl<sub>2</sub> at final reaction conditions. If higher MgCl<sub>2</sub> concentrations are desired, use 50 mM MgCl<sub>2</sub> solution to increase the MgCl<sub>2</sub> conc.

**SciPhi™ DMSO**- 100% with freezing point 18-19°C

### Instruction for Use

1. Thaw at room temperature then gently vortex and briefly centrifuge all solution.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 μl reaction volume.

Component	Reaction Conc./Vol.
SciPhi™ 5X Hi-Fi buffer	10 μL
dNTP Mix (10 mM each)	1 μL
Forward primer	0.1-1.0 μM
Reverse primer	0.1-1.0 μM
Template DNA	1pg- 10 ng (complex DNA) 20-250 ng (genomic DNA)
DMSO (optional)	1.5 μl
SciPhi™ High-fidelity DNA Polymerase	0.5 μl
SciPhi™ Nuclease-free Water (NXG331)	Up to 50 μL
<b>Total volume</b>	<b>50 μL</b>

3. Gently vortex the samples and spin down.
4. Place the reaction in a thermal cycler, perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temp.	Time	Number of cycles
Initial denaturation	98°C	30 s	1
Denaturation	98°C	5-10 s	25-35
Annealing	T <sub>m</sub>	10- 30 s	
Extension	72°C	15-30 s/kb	
Final Extension	72°C	5-10 min	1

### Points need to be considered

- Addition of DMSO is recommended for GC-rich amplicons. DMSO is not recommended for amplicons with very low GC % or amplicons that are > 20 kb.
- When using higher concentrations of DMSO, it is important to reduce the annealing temperature, as DMSO lowers the melting point of primers. Studies have shown that 10% DMSO decreases the annealing temperature by approximately 5.5–6.0°C
- For optimal performance, it is essential to use high-quality dNTPs. Avoiding dUTP-derivatives or dITP in the template or primers is advisable since the polymerase cannot effectively utilize these analogues. It is recommended to consistently use 200 μM of each dNTP for best results

### Troubleshooting:

For troubleshooting, please email us at [techteam@nextgenlife.com](mailto:techteam@nextgenlife.com); [info@nextgenlife.com](mailto:info@nextgenlife.com).

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