Solis Hot Start HiFi DNA Polymerase Kit

Catalogue Number	Pack Size (1 U/µl)
01-16-KIT-0000S	25 U
01-16-KIT-00100	100 U
01-16-KIT-00500	500 U

Shipping:

At room temperature.

Store at –20°C upon receipt

Batch Number and Expiry Date:

See vial.

Storage and Stability*:

- Routine storage at –20°C (–28°C to –18°C) until expiry date.
- Stable at room temperature (25°C) for 1 month.
- Freeze-thaw stability:15 cycles.

Reaction setup:

At room temperature.

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

Product description:

- Solis Hot Start HiFi DNA Polymerase is a modified polymerase with enhanced fidelity of >100x compared to wild type *Taq* polymerase.
- Suitable in applications requiring high fidelity PCR, such as sequencing or cloning. Additionally, enhanced processivity allows for long range amplification up to 12 kb from genomic DNA and 24 kb from Lambda DNA. Increased GC tolerance allows robust amplification of challenging amplicons with GC content ranging between 30-79% when combined with the 10x GC-rich Enhancer.
- Solis Hot Start HiFi DNA Polymerase is engineered to efficiently incorporate dUTPs and is well suited for amplifying bisulfite treated DNA, damaged DNA (e.g., FFPE samples) or for prevention of carryover contamination (when used with dUTPs and UNG).
- Solis Hot Start HiFi DNA Polymerase is a chemically modified DNA polymerase, enabling hot-start PCR that improves specificity and accuracy, minimizes mispriming and extension from non-specifically annealed primers and primer-dimers. Solis Hot Start HiFi DNA Polymerase is inactive at room temperature and is activated by an initial activation step for 15 min at 95°C.
- Solis Hot Start HiFi DNA Polymerase possesses 5′→3′ polymerase and 3′→5′ exonuclease (proofreading) activity with no 5′→3′ exonuclease activity.

Kit Content:

Component	Catalogue Number		
	01-16-KIT-0000S	01-16-KIT-00100	01-16-KIT-00500
Solis Hot Start HiFi DNA Polymerase (1 U/µl)	25 U / 25 µl	100 U / 100 µl	500 U / 500 µl
5x HiFi Reaction Buffer	250 µl	1 ml	5 ml
10x GC-rich Enhancer	0.5 ml	0.5 ml	3 ml

- **Solis Hot Start HiFi DNA Polymerase (1 U/µl)** in enzyme dilution buffer.
- **5x HiFi Reaction Buffer** with 10 mM $MgCl_2$ (2 mM in 1x concentration).
- **10x GC-rich Enhancer** is an additive that facilitates amplification of difficult templates (e.g., GC-rich DNA templates).

  This solution should be used at a defined final concentration (1x or 2x solution). 10x GC-rich Enhancer is NOT a reaction buffer.

Additional reagents required:

- Template DNA
- Gene-specific primer pair
- dNTP Mix (20 mM of each, Cat. No. 02-31-00020)
- Nuclease-free PCR Grade Water (Cat. No. water-025)

Step-by-step guidelines:

1. Thaw the reagents at room temperature. Mix each reagent by gentle vortexing or pipetting up and down, then spin down.

2. Prepare a reaction mix at room temperature. Add all required components except the template DNA.

Component	Volume[1]	Final conc.
5x HiFi Reaction Buffer	5 µl	1x
dNTP Mix (20 mM of each)	0.38 µl	300 µM of each
Forward Primer (10 µM)	0.75 µl	300 nM
Reverse Primer (10 µM)	0.75 µl	300 nM
Solis Hot Start HiFi DNA Polymerase (1 U/µl)	0.5 µl[2]	0.02 U/µl[2]
10x GC-rich Enhancer (optional)	2.5 µl	1x
Template DNA (added at step 4)	Variable[3]	Variable[3]
Nuclease-free water	up to 25 µl	
Total reaction volume	**25 µl**	

[1] Scale all components proportionally according to sample number and reaction volumes. Make sure you use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors.

[2] 0.02 U/µl is the recommended final concentration. If necessary, optimize by increasing the enzyme concentration up to 0.05 U/µl. For highly multiplexed reactions higher enzyme concentration is recommended.

[3] For low complexity templates (i.e. plasmid, lambda), use 1 pg–1 ng of DNA per 25 µl reaction. For higher complexity templates (i.e. gDNA), use 1 ng–100 ng of DNA per 25 µl reaction.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells or tubes.

4. Add template DNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used and centrifuge the reactions briefly.

5. Incubate your PCR reactions in thermal cycler as follows.

Step	Temperature	Time	Cycles
Initial activation[1]	95°C	12-15 min	1
Denaturation[2]	95°C	30 sec	25–35
Annealing[3]	55-68°C	30 sec	
Extension[4]	72°C	45–60 sec/kb[4]	
Final extension[4]	72°C	2 min[4]	1

[1] Initial incubation at 95 °C for 12-15 min is needed for the activation of polymerase and denaturation of template DNA.

[2] For high GC content amplicons use denaturation step at 98°C for 20 seconds.

[3] The annealing temperature depends on the melting temperature of the primers.

[4] Extension and final extension time depends on the length of the fragment to be amplified. A time of 1 min/kb is recommended e.g., for 5 kb fragment use 5 minutes.

Recommendations for a successful PCR experiment

Prerequisites for a successful PCR include the design of optimal primers, the use of high-quality template DNA and appropriate concentrations of reaction components.

Use dedicated software, such as Primer3 and NCBI Primer-BLAST to design target-specific primers. The optimal primer length is 20–30 bp, with GC-content 35–65% and calculated melting temperatures (T_m) 60–70°C. T_m of the two primers should not differ by more than 3°C. Analyze your primers for self-complementarity

and stable secondary structures, presence of secondary structures increases probability of mis-priming and primer-dimers formation.

The integrity, purity and concentration of the DNA template should be suitable for the PCR experiment. Always include a no-template control (NTC) by replacing the DNA template with the same volume of nuclease-free water.

Troubleshooting Guide

No or low PCR yield

- Solis Hot Start HiFi DNA Polymerase (1 U/µl) was not activated – make sure that your PCR starts with an initial incubation for 12-15 min at 95 °C.
- Cycling conditions are not optimal – adjust annealing temperature (T_a); if needed determine the optimal T_a by running a temperature gradient and determine the optimal annealing time between 30-60 sec; adjust denaturation temperature and time, use 98°C for 20 sec for high GC content amplicons; increase the extension time (if amplifying a long target); increase the number of cycles by 3–5.
- Poor quality of template – check the template's purity and integrity, ensure that your template doesn't contain PCR inhibitors.
- Template concentration is too low – increase the concentration of DNA template in a reaction by increasing sample volume; or concentrate the DNA by precipitation before adding the sample to the reaction.
- Primer concentration is not optimal – titrate primer concentration (final concentration 100–300 nM of each); ensure that both primers have the same concentration.

- Reaction components are degraded – check the storage conditions and expiry date of the reagents; perform a positive control with template DNA and/or reagents previously known to amplify.

Non-specific products

- Non-specific amplification – ensure that your primers are target-specific.
- Primer concentration is not optimal – titrate primers (final concentration 100–300 nM of each); too high primer concentration can reduce the binding specificity, resulting in unwanted products.
- Primer annealing temperature (T_a) is too low – increase the T_a; keep your primer T_a 2–5°C below the T_m of the primer having the lowest T_m.
- Too many cycles – reduce the cycle number by 3–5.
- Contamination – to avoid contamination, work in dedicated space, keep pre- and post-amplification areas separate, use personal protective equipment, decontaminate your surfaces and equipment, if possible, aliquot your reagents into smaller volumes to prevent contamination of stock solutions.

Smearing in electrophoresis

- Too much template – load lower amount or prepare serial dilutions of template.
- Too many cycles – reduce the cycle number by 3–5.
- Extension time is too long – reduce extension time.
- Primer design is not optimal – review your primers and redesign the primers if needed.
- Enzyme concentration is too high – decrease the amount of enzyme in final solution by 0.005 U/µl increments (optimal enzyme concentration in final PCR solution is 0.02–0.05 U/µl).

Unit definition:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 74°C.

Safety precautions:

Please refer to the Safety Data Sheet for more information.

Technical support:

Contact your sales representative for any questions or send an email to support@solisbiodyne.com.

DS-01-16-KIT v2. Effective from 20.02.2025

Reason for revision: Information regarding polymerase properties updated.

*__Product stability__ is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. The customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage and handling of the assay assembled and packed by the customer.

Permitted Use: This product is supplied for research use only. Some applications of this product may require a license/licenses from Solis BioDyne OÜ or one or more third parties which are not provided by the purchase of this product. This product shall comply with its relevant specification and be fit for its stated purpose, Solis BioDyne OÜ gives no other warranty and makes no representation as to description or quality. For more information and full disclaimers, please contact our customer service. **Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

Manufacturer: Solis BioDyne OÜ | Teaduspargi 9, 50411 | Tartu, Estonia (EU)