HOT FIREPol® DNA Polymerase Kit

Catalogue Number	Pack Size (5 U/µl)
01-02-KIT-0000S	100 U
01-02-KIT-00500	500 U
01-02-KIT-01000	1000 U

Store at –20°C upon receipt

Shipping:

At room temperature.

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 4°C (2°C to 8°C) for 6 months.
- Stable at room temperature (25°C) for 1 month.
- Freeze-thaw stability: 30 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:

- HOT FIREPol® is a chemically modified FIREPol® DNA Polymerase (see pg. 3) enabling hot-start PCR that improves specificity and accuracy, minimizes mispriming and extension from non-specifically annealed primers and primer-dimers.
- HOT FIREPol® is inactive at room temperature and is activated by an initial activation step for 12–15 min at 95°C.
- Recommended for routine applications (fragment up to 3 kb from genomic DNA).
- Possesses 5′→3′ polymerase and 5′→3′ exonuclease activity, as well as a non-template-dependent terminal transferase activity, but lacks a 3′→5′ exonuclease (proofreading) activity making the generated product suitable for TA-cloning.
- The fidelity of HOT FIREPol® is similar to a regular *Taq* DNA Polymerase (error rate per nucleotide ca 2.5 x10^{-5}).

Kit Content:

Component	Catalogue Number		
	01-02-KIT-0000S	01-02-KIT-00500	01-02-KIT-01000
HOT FIREPol® DNA Polymerase (5 U/µl)	100 U / 20 µl	500 U / 100 µl	1000 U / 200 µl
HOT FIREPol® 10x Buffer B1	500 µl	2.5 ml	5.0 ml
HOT FIREPol® 10x Buffer B2	500 µl	2.5 ml	5.0 ml
25 mM $MgCl_2$	500 µl	2.5 ml	5.0 ml
10x GC-rich Enhancer	100 µl	100 µl	500 µl

- **HOT FIREPol® DNA Polymerase** (5 units/µl) in 20 mM Tris-HCl pH 8.7 at 25°C, 100 mM KCl, 0.1 mM EDTA, 50% glycerol (v/v), and stabilizers.
- **HOT FIREPol® 10x Buffer B1** (Mg^{2+} and detergent free): 0.7 M Tris-HCl, 0.175 M $(NH_4)_2SO_4$.
- **HOT FIREPol® 10x Buffer B2** (Mg^{2+} free, with detergent): 0.7 M Tris-HCl, 0.175 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20.

  HOT FIREPol® 10x Buffer B2 contains non-ionic detergent suppressing inhibitory effects of the trace of DNA extraction buffers and enhancing PCR yield and efficiency.

- **25 mM $MgCl_2$**
- **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, 2x or 3x solution). 10x GC-rich Enhancer is NOT a reaction buffer and should be used ONLY IF non-specific amplification occurs.

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)

Footnote: FIREPol® is a highly processive, thermostable DNA Polymerase. Due to its genetic modifications**, it has an enhanced stability at room temperature with no activity loss for up to 1 month. It is produced in *E. coli* strain that carries an overproducing plasmid with a modified gene of *Thermus aquaticus* DNA Polymerase.

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.
HOT FIREPol® DNA Polymerase (5 U/µl)	0.08–0.2 µl	0.02–0.05 U/µl
HOT FIREPol® 10x Buffer B1 or B2	2 µl	1x
25 mM $MgCl_2$	1.2–2 µl	1.5–2.5 mM
dNTP Mix (20 mM of each)	0.2 µl	200 µM of each
Forward Primer (10 µM)	0.2–0.6 µl	100–300 nM
Reverse Primer (10 µM)	0.2–0.6 µl	100–300 nM
10x GC-rich Enhancer (optional)	2, 4 or 6 µl	1x, 2x or 3x
Template DNA (added at step 4)	Variable	Variable[2]
Nuclease-free water	up to 20 µl	
Total reaction volume	**20 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] For low complexity templates (i.e. plasmid, lambda), use 20 pg–2 ng of DNA per 20 µl reaction. For higher complexity templates (i.e. gDNA), use 2 ng–200 ng of DNA per 20 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	95°C	15–30 sec	26–35
Annealing[2]	50–68°C	30–60 sec	
Extension[3]	72°C	45 sec–4 min	
Final extension	72°C	5–10 min	1

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

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

[3] Extension time depends on the length of the fragment to be amplified. A time of 1 min/kb is recommended.

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.

Please see the Troubleshooting Guide below for suggestions and help with specific problems.

Troubleshooting Guide

No or low PCR yield

- HOT FIREPol® DNA Polymerase 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; 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-01-02-KIT v2. Effective from 02.04.2024

Reason for revision: Kit component 10x Solution S changed to 10x GC-rich Enhancer.

*__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.

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Trademark information: FIREPol is a registered trademark of Solis BioDyne OÜ.

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