

FIREPol® DNA Polymerase (5 U/μl)

Catalogue Number	Pack Size (5 U/μl)
01-01-0000S	100 U
01-01-00500	500 U
01-01-01000	1000 U
01-01-02000	2000 U

Shipping:

At room temperature

Batch Number and Expiry Date:

See vial

Stability at room
temperature
1 MONTH

Store at **-20 °C**

Storage and Stability:

- Routine storage at -20 °C until expiry date
- Can be stored at +4 °C for up to 6 months
- Stability at room temperature (15–25 °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.

DS-01-01 v1

1/8

- **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.
- **FIREPol® 10x Buffer B** (Mg²⁺ free, with detergent): 0.8 M Tris-HCl, 0.2 M (NH₄)₂SO₄, 0.2% w/v Tween-20.

FIREPol® 10x Buffer B contains non-ionic detergent suppressing inhibitory effects of the traces of DNA extraction buffer and enhancing PCR yield and efficiency.

- **FIREPol® 10x Buffer BD** (Mg²⁺ and detergent free): 0.8 M Tris-HCl, 0.2 M (NH₄)₂SO₄.
- **25 mM MgCl₂**
- **10x Solution S** 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 Solution S 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)

Product description:

- 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.
- Recommended for routine applications (fragment up to 3 kb from genomic DNA).
- Possesses 5'→3' polymerase and 5'→3' endonuclease 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 FIREPol® is similar to a regular Taq DNA Polymerase (error rate per nucleotide ca 2.5 x10⁻⁵).

Contents:

Component	Catalogue Number			
	01-01-0000S	01-01-00500	01-01-01000	01-01-02000
FIREPol® DNA Polymerase (5 U/μl)	100 U / 20 μl	500 U / 100 μl	1000 U / 200 μl	2000 U / 400 μl
FIREPol® 10x Buffer B	500 μl	2.5 ml	5.0 ml	2 x 5.0 ml
FIREPol® 10x Buffer BD	500 μl	2.5 ml	5.0 ml	2 x 5.0 ml
25 mM MgCl₂	500 μl	2.5 ml	5.0 ml	2 x 5.0 ml
10x Solution S	100 μl	100 μl	500 μl	500 μl

2/8

Step-by-step guidelines:

1. Thaw the reagents at room temperature. Mix each reagent by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Prepare a reaction mix at room temperature. Add all required components except the template DNA.

Component	Volume ¹	Final conc.
FIREPol® DNA Polymerase (5 U/μl)	0.08–0.2 μl	0.02–0.05 U/μl
FIREPol® 10x Buffer B or BD	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 Solution S (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.

- Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells.
- 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.
- Incubate your PCR reactions in thermal cycler as follows.

Step	Temperature	Time	Cycles
Initial denaturation¹	95 °C	3–5 min	1
Denaturation	95 °C	15–30 sec	26–35
Annealing ²	50–68 °C	30–60 sec	
Extension ²	72 °C	45 sec–4 min	
Final extension	72 °C	5–10 min	1

¹ Complex templates, such as gDNA, require longer time to denature (5 min). With low complexity templates (i.e. lambda, plasmid DNA), initial denaturation time can be reduced to 3 min.

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

5/8

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

- Cycling conditions are not optimal – decrease the primer 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.
- 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

6/8

control with template DNA and/or reagents previously known to amplify.

Non-specific products

- Non-specific amplification – use hot-start PCR enzyme (e.g. HOT FIREPol DNA Polymerase, Cat. No. 01-02-00500); ensure that your primers are target-specific.
- Primer concentration is not optimal – titrate primer concentration (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 annealing temperature 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, 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/ μ l increments (optimal polymerase concentration in final PCR solution is 0.02–0.05 U/ μ l).

7/8

Source:

E. coli strain that carries an overproducing plasmid with a modified gene of *Thermus aquaticus* DNA Polymerase.

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.

Quality control:

The enzyme is free of nicking and priming activities, exonucleases and non-specific endonucleases. SDS/PAGE 95 kD band, >98% pure. Activity and stability tested via thermocycling. The error rate per nucleotide per cycle is ca 2.5×10^{-5} ; the accuracy is ca 4×10^4 . Estimated half-life at 95 °C is 1.5 hours.

Safety precautions:

Please refer to Safety Data Sheet for more information.

Technical support:

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

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This product is supplied for research use only. Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

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8/8