Solis BioDyne
Data Sheet

HOT FIREPol® DNA Polymerase

(HOT START DNA Polymerase)

Cat. No.	Pack Size	Conc.
01-02-0000S	100 U SAMPLE	5 U/µl
01-02-00500	500 U	5 U/µl
01-02-01000	1000 U	5 U/µl

For *in vitro* use only.

Description:

HOT FIREPol® DNA Polymerase is a chemically modified FIREPol® DNA Polymerase. At ambient temperatures it is inactive, having no polymerization activity. HOT FIREPol® DNA Polymerase is activated by a 15 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR setup. The enzyme has 5'→ 3' polymerase and 5' flap endonuclease activity.

Source:

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

Applications:

- Hot Start PCR
- DHPLC
- TA cloning

Reagents Provided:

- **HOT FIREPol® DNA Polymerase**
- **HOT FIREPol® 10x Buffer B1** (Mg^{2+} and detergent free) *Tris-HCl and $(NH_4)_2SO_4$*
- **HOT FIREPol® 10x Buffer B2** (Mg^{2+} free) *Tris-HCl, $(NH_4)_2SO_4$ and detergent*
- **25 mM $MgCl_2$**
- **10x Solution S**
  Additive that facilitates amplification of difficult templates (e.g. GC-rich DNA templates). This solution should be used at a defined working concentration (1x, 2x or 3x solution).
  Solution S is NOT a reaction buffer and should be used ONLY IF non-specific amplifications occur.

Concentration:

5 U/µl

Unit definition:

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

Storage and Dilution buffer:

50% glycerol (v/v), 20 mM Tris-HCl pH 8.7 at 25ºC, 100 mM KCl, 0.1 mM EDTA and stabilizers.

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 thermo cycling. The error rate per nucleotide per cycle is ~ 2.5 x 10^{-5}; the accuracy is ~ 4 x 10^{4}. Estimated half life at 95ºC is 1.5 hours.

Shipping and Storage conditions:

Routine storage: -20ºC
Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of HOT FIREPol® DNA Polymerase.

Recommended PCR Buffers:

Our standard buffers are:

- **HOT FIREPol® 10x Buffer B1** (Mg^{2+} and detergent free) *Tris-HCl and $(NH_4)_2SO_4$*
- **HOT FIREPol® 10x Buffer B2** (Mg^{2+} free) *Tris-HCl, $(NH_4)_2SO_4$ and detergent*

For further optimization please try our whole set of buffers available upon request:

- **10x Reaction buffer B** (Mg^{2+} free) *0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20*
- **10x Reaction buffer BD** (Mg^{2+} and detergent free) *0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$*

Recommendations:

Reaction setup at room temperature is highly recommended for HOT FIREPol® DNA Polymerase.

In order to prevent contamination, we recommend you to setup the reaction under laminar or in PCR box.

Recommended PCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® (5 U/µl)	0.4-1.0 µl	0.02-0.05 U/µl (2-5 U)
10x Buffer B1/B2,B/BD	10 µl	1x
25 mM $MgCl_2$	6-10 µl	1.5-2.5 mM
dNTP mix (20 mM of each)	1 µl	200 µM of each
Primer Forward (10 pmol/µl)	1-3 µl	0.1-0.3 µM
Primer Reverse (10 pmol/µl)	1-3 µl	0.1-0.3 µM
DNA template[1]	variable[1]	variable[1]
10x Solution S ***Not for standard PCR***	0, 10, 20 or 30 µl	1x, 2x or 3x
H_2O PCR grade	Up to 100 µl	
Total	**100 µl**	

[1]Conc. of cDNA 0.01 pg/µl -0.1 ng/µl ; gDNA 0.1 ng/µl – 10 ng/µl

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Recommended PCR cycles:

Cycle step	Temp.	Time	Cycles
Initial activation[2]	**95ºC**	**12-15 min**	1
Denaturation	95ºC	15-30 s	26-35
Annealing	50-68ºC	30-60 s	
Elongation[3]	72ºC	1-4 min	
Final elongation	72ºC	5-10 min	1

[2]To activate the polymerase, include an incubation step **at 95ºC for 12 - 15 minutes** at the beginning of the PCR cycle.

[3]Elongation time should be ~1 min/1 kb.

Safety warnings and precautions:

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Related products:

Product name	Pack size	Cat. No.
FIREPol® DNA Polymerase	500 U	01-01-00500
FIREPol® DNA Polymerase	1000 U	01-01-01000
FIREPol® DNA Polymerase	2000 U	01-01-02000
5x FIREPol® Master Mix *(1.5 mM $MgCl_2$ final conc.)*	250 reactions	04-11-00115
5x FIREPol® Master Mix *(2.5 mM $MgCl_2$ final conc.)*	250 reactions	04-11-00125
5x FIREPol® Master Mix Ready to Load *(1.5 mM $MgCl_2$ final conc.)*	250 reactions	04-12-00115
5x FIREPol® Master Mix Ready to Load *(2.5 mM $MgCl_2$ final conc.)*	250 reactions	04-12-00125
dNTP MIX *(20 mM of each)*	20 µmol	02-31-00020
dNTP MIX *(20 mM of each)*	100 µmol	02-31-00100
dNTP SET *(100 mM)*	4 x 25 µmol	02-21-00100
dNTP SET *(100 mM)*	4 x 100 µmol	02-21-00400