Solis BioDyne
Data Sheet

FIREPol® DNA Polymerase Core Kit

with positive control and loading dyes

Cat. No.	Pack Size	Conc.
01-23-00500	500 U	5 U/µl

For *in vitro* use only

Description:

FIREPol® DNA Polymerase Core Kit with positive control and loading dye includes necessary reagents for 200-500 amplification reactions, lambda DNA template for positive control, primers mix for a 500 bp amplicon and two loading dyes that allow to monitor progress during electrophoreses.

Applications:

- Suited for a wide range of PCR assays
- TA cloning

Reagents Provided:

Component	Volume
FIREPol® DNA Polymerase (5 U/µl)	500U
10x Buffer B	2 x 1,2 ml
10x Buffer BD	2 x 1,2 ml
25 mM $MgCl_2$	2 x 1,2 ml
10x Solution S	0,5 ml
20 mM dNTP mix	20 µmol
500 bp Primer Mix 25 µM each	40µl
Lambda DNA template (0,5 ng/µl)	20µl
6x DNA Loading Dye Buffer Double Blue	3 x 1 ml

Component specifications:

FIREPol® DNA Polymerase (5 U/µl) is a highly processive, thermostable DNA polymerase. Due to its genetic modifications FIREPol® has an enhanced stability at room temperature with no activity loss for up to 1 month. The enzyme has 5'→3' polymerization-dependent exonuclease replacement activity but lacks 3'→ 5' exonuclease activity.
Purified from an E.coli strain that carries an overproducing plasmid containing 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 74ºC.

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.

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.

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 FIREPol® DNA Polymerase.

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 $(NH_4)_2SO_4$

25 mM $MgCl_2$

10 x 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.

dNTP Mix
This ready-to-use mix consists of dATP, dCTP, dGTP and dTTP (each at a final concentration of 20 mM) in TE buffer, within one vial. The total concentration is 80 mM.

Lambda DNA template
Bacteriophage lambda DNA template is for positive control. The concentration is 0.5 ng/ µl and it is stored in TE buffer

500 bp Primer mix (25 µM each)

3 x 1ml DNA Loading dye Double Blue (TRIS-HCl, EDTA and Ficoll)
6x DNA Loading Dye Buffer Double Blue can be used for loading DNA samples (PCR products, restriction fragments) on agarose or polyacrylamide gel. In 1% agarose gel Bromophenol Blue comigrates with ~300 bp (1 x TBE) or ~400 bp (1 x TAE) fragments and Xylene Cyanole FF comigrates with ~3500 bp (1 x TBE) or ~5000 bp (1 x TAE) fragments.

Solis BioDyne
Riia 185a, 51014 Tartu, Estonia, tel: +372 740 9960, fax: +372 740 2079, e-mail: solis@sbd.ee, www.sbd.ee

Recommended PCR reaction mix:

Component	Volume	Final conc.
FIREPol® DNA Polymerase (5 U/µl)	0.2-0.5 µl	0.02-0.05 U/µl (1-2,5 U)
10 x Buffer B or BD	5 µl	1x
25 mM $MgCl_2$	3-5 µl	1.5-2.5 mM
20 mM dNTP mix	0.5 µl	200 µM
Primer Forward (10 pmol/µl)	0.5-1.5 µl	0.1-0.3 µM
Primer Reverse (10 pmol/µl)	0.5-1.5 µl	0.1-0.3 µM
DNA template	X µl	0.01-10 ng/µl
10 x Solution S ***Not for standard PCR***	0, 5, 10 or 15 µl	1x, 2x or 3x
H_2O PCR grade	Up to 50 µl	
Total	**50 µl**	

Recommended PCR reaction mix for amplifying control template:

Component	Volume	Final conc.
FIREPol® DNA Polymerase (5 U/µl)	0.2-0.5 µl	0.02-0.05 U/µl (1-2,5 U)
10 x Buffer B or BD	5 µl	1x
25 mM $MgCl_2$	3-5 µl	1.5-2.5 mM
20 mM dNTP mix	0.5 µl	200 µM
500bp Primer Mix Mix (25 µM each)	0.5-1.5 µl	0.1-0.3 µM
Lambda DNA template	1 µl	0,01 ng/µl
H_2O PCR grade	Up to 50 µl	
Total	**50 µl**	

Related products:

Product name	Pack size	Cat. No.
HOT FIREPol® DNA Polymerase	500 U	01-02-00500
HOT FIREPol® DNA Polymerase	1000 U	01-02-01000
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

Recommended PCR cycles:

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

IMPORTANT: Annealing temperature should be 2-6ºC lower than the primer melting temperature. 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.

Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required.

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