vivantis

DNA AMPLIFICATION PRODUCT

Product Datasheet

Pfu DNA Polymerase

Product No : PL5201
Quantity : 100u

Lot :
Expiry Date :
Concentration : 5u/µl
Supplied with : 2ml of 10X ViBuffer A
1ml of 10X ViBuffer S
1ml of 50mM $MgCl_2$

Store at - 20°C

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Description:

Pfu DNA Polymerase is an extremely thermostable proofreading DNA polymerase. It is suitable for applications requiring high temperature synthesis of DNA. *Pfu* DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5' to 3' direction with the presence of Mg^{2+}. It exhibits the 3' to 5' proofreading activity, resulting in over 10-fold higher fidelity than possible with *Taq* DNA Polymerases.

Features:

- Ultra pure recombinant protein allows amplification up to 8kb.
- Recommended for use in high-fidelity amplification, amplification of GC-rich sequences or problematic secondary structures, primer extension reactions at elevated temperatures and cloning of blunt-ended amplification products.

Unit Definition :

1u is defined as amount of enzyme that required to catalyze the incorporation of 10nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Reaction Buffer:

10X ViBuffer A (*without* $MgCl_2$)**:**
500mM KCl, 100mM Tris-HCl (pH9.1 at 20°C), 0.1% Triton™X-100. The buffer is optimized for use with 0.1-0.2mM of each dNTP.

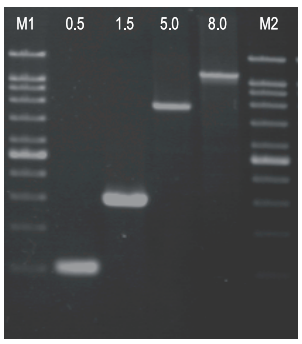
10X ViBuffer S:
160mM $(NH_4)_2SO_4$, 500mM Tris-HCl (pH 9.2 at 22°C), 17.5mM $MgCl_2$ and 0.1% Triton™X-100. The buffer is optimized for use with 0.35mM of each dNTP.

Storage Buffer:

20mM Tris-HCl (pH 8.0 at 22°C), 100mM KCl, 0.5% Tween™ 20, 0.5% Nonidet P-40, 0.1mM EDTA, 1mM DTT and 50% glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.



Amplification Using Vivantis *Pfu* DNA Polymerase

Lane M1 : VC 1kb DNA Ladder

Lane 0.5 and 1.5kb : 0.5kb PCR amplification product generated using 0.2mM dNTPs and 2.0u Vivantis *Pfu* DNA Polymerase.

Lane 5kb and 8kb : 5kb and 8kb amplification products generated using 0.25mM dNTPs, 2.5u Vivantis *Pfu* DNA Polymerase and 3% of formamide.

Lane M2 : VC Lambda/*Hind*III Marker

0.7% TAE agarose gel

DSPL5201_rev0_010311

SUGGESTED INITIAL PCR CONDITIONS FOR VARIOUS PCR PRODUCT SIZES WITH VIVANTIS DNA POLYMERASES (#PL5201 - 06 / #PL5211 - 16)

REACTION MIX (FINAL CONCENTRATION) :

Product Size	100bp - 5kb	5kb - 8kb
dNTP Mix	100µM	200µM
ViBuffer (1X)	A	S
Ultrapure DMSO	-	3%
DNA Polymerase	Refer to the below Table (A)	

Primers : 0.5µM
Template: Plasmid (0.02-0.2ng)
Genomic (0.05-5µg)

Top up with sterile dH_2O to 50 ml.

Product Size	100bp - 5kb	5kb - 8kb
Denaturation	95°C, 2 min	95°C, 2 min
Denaturation	94°C, 20 s	94°C, 12 s
Annealing*	50 - 68°C, 30 s	50 - 68°C, 30 s
Extension / 1kb	72°C, 2 min	72°C, 2 min
Cycles	25 - 35	25 - 35
Final Extension	72°C, 7 min	72°C, 7 min

*Primer dependent

TABLE (A) : RECOMMENDED UNITS FOR SPECIFIC VIVANTIS DNA POLYMERASES PER 50µL REACTION VOLUME :

Product Size	*Taq* (#PL1201 - 06)[1]	Max *Taq* (#PL2201 - 06)[2]	*Pfu* (#PL5201 - 06)	Max *Pfu* (#PL5211 - 16)
0.1 - 5.0kb	2.0	2.0	2.0	2.0
5.0 - 8.0kb	2.5	2.0	2.5	2.0
8.0 - 20.0kb	2.5	2.0	2.5	2.0
>20.0kb	-	2.0	-	2.0

* This protocol is subjected to changes depending on the template DNA

[1] Also applied for At *Taq* (#PL3201-06)
[2] Also applied for Max At *Taq* (#PL4201-06)

Product Use Limitation
This product is for research purpose an *in vitro* use only

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