Hot Start *Pfu* DNA Polymerase

Intact Genomics®

Catalog #	3316	3318
Package Size	200 units	1,000 units
Volume	40 µl	200 µl
Concentration	5 units/µl	

Catalog #	3316d	3318d
Package Size	200 units	1,000 units
Volume	40 µl	200 µl
Concentration	5 units/µl	

*Catalog numbers ending with "d" include separate dNTP mix.

Description

Hot start *Pfu* DNA polymerase is formulated with chemical modification which effectively neutralize 5´→ 3´ DNA polymerase and 3´→ 5´ exonuclease (proofreading) activities at room temperature, but regain the full enzyme activity upon the initial denaturation step. Hot start *Pfu* DNA polymerase retains the high fidelity, sensitivity and processivity of Pfu DNA polymerase, while provides reduced background by facilitating room temperature PCR assembly and preventing priming until stringent primer annealing temperatures are reached. *Pfu* DNA polymerase has an error rate six-fold lower than *Taq* DNA polymerase, and significantly lower than the error rates of most other proofreading enzymes or DNA polymerase mixtures (1). This product is supplied with the unique Intact Genomics 10x PCR reaction buffer, containing $MgCl_2$, which produces a final Mg2+ concentration of 1.5 mM, and 5x Magic Enhancer that enables efficient amplification of GC rich templates up to 84%.

Protein Purity

The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

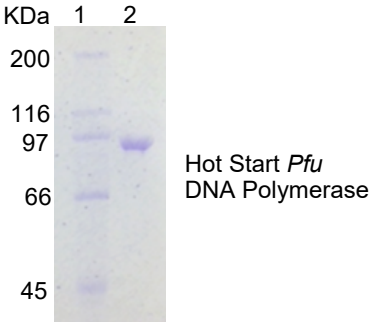

Fig. 1: Lane 1. Protein Marker
Lane 2. Hot Start *Pfu* DNA Polymerase

Product Source

E. coli strain expressing a *Pfu* DNA Polymerase gene from Pyrococcus furiosus.

Hot Start *Pfu* Polymerase Comparison Data

A typical gel data with hot start *Pfu* Polymerase for PCR assay is shown below in Fig. 2.

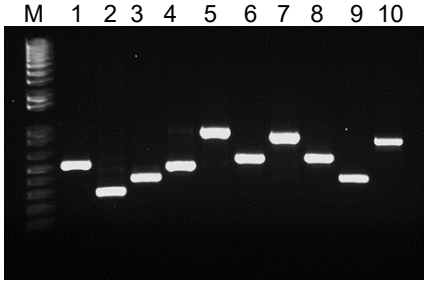

Fig. 2: Lane M. DNA Marker
Lane 1-10. Different PCR products

Applications

- Routine PCR
- Primer extension
- Colony PCR
- Genotyping
- Efficient for amplifying high GC content template DNA with Magic Enhancer

Product Includes

1) Hot Start *Pfu* DNA Polymerase
2) 10x PCR Buffer with Mg^{2+}
3) 5x Magic Enhancer
4) 10 mM dNTP Mix (Cat. # 3316d, 3318d only)

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

10X PCR Buffer with Mg^{2+}

100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$, 100 mM KCl , 80 mM $(NH_4)_2SO4$, 0.5% Igepal CA 630

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP into acid-insoluble form in 30 minutes at 72 ºC.

Protocol

1. Thaw 10x PCR Buffer, dNTP mix, primer solutions, 5X Magic Enhancer (if required) and mix thoroughly before use.
2. Prepare a reaction mix according to the following table: The reaction mix typically contains all the components needed for PCR except the template DNA.

PCR Reaction Set Up:	
Template DNA	x µl (0.01-0.5 µg)
10x PCR Buffer	10.0 µl
dNTP (10 mM)	2.0 µl
Forward Primer	x µl (0.1- 0.5 µM)
Reverse Primer	x µl (0.1- 0.5 µM)
5x Magic Enhancer (optional)	20 µl
Hot start *Pfu* DNA Polymerase (5 U/µl)	0.5 µl
H_2O up to	100.0 µl

3. Mix the reaction mixture thoroughly.

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4. Add template DNA to the individual PCR tubes containing the reaction mixture.
5. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in the following table.

Note: PCR program must start with an initial heat-activation step at 95 °C for 15 min.

PCR Cycling Conditions:			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	95 ºC	15 min	1
Denaturation	94 ºC	30-60 sec	25-35
Annealing	52-66 ºC	30-60 sec	
Extension	72-74 ºC	1-2 min	
Final extension	72-74 ºC	10 min	1
Hold	4-12 ºC	∞	

6. Place the PCR tubes in the thermal cycler and start the cycling program.

Reference

1. Frey, B. and Suppman, B. (1995). BioChemica. 2, 34-35.