Intact Genomics®

Pfu DNA Polymerase

Catalog #	3312	3314
Package Size	200 units	1,000 units
Volume	40 µl	200 µl
Concentration	5 units/µl	

Catalog #	3312d	3314d
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

Pfu DNA polymerase is a heat stable DNA polymerase which has 5´→ 3´ DNA polymerase and 3´→ 5´ exonuclease (proofreading) activities. *Pfu* DNA polymerase retains the high fidelity, sensitivity and processivity with 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 Mg^{2+} 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).

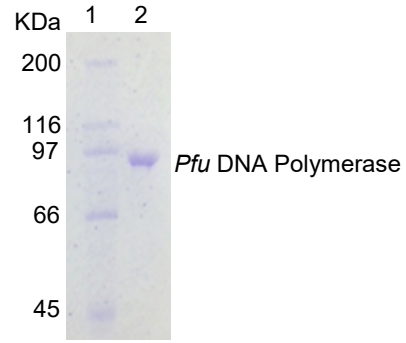


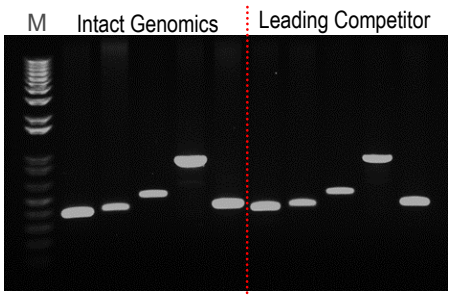
Fig. 1: Lane 1. Protein Marker
Lane 2. *Pfu* DNA Polymerase

Product Source

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

Pfu DNA Polymerase Comparison Data

We repeatedly compare our *Pfu* Polymerase side-by-side with a leading competitor for PCR assay. Our enzyme is better than the competitor. A typical gel picture is shown below:



Applications

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

Product Includes

1) *Pfu* DNA Polymerase
2) 10x PCR Buffer with Mg^{2+}
3) 5x Magic Enhancer
4) 10 mM dNTP (Cat. # 3312d, 3314d 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
Pfu DNA Polymerase (5 U/µl)	0.5 µl
H_2O up to	100.0 µl

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3. Mix the reaction mixture thoroughly.
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.

PCR Cycling Conditions:			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	95 ºC	3-5 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.