

# Nova Pfu DNA Polymerase

Without dNTPs

Reference: AB12027; AB12028; AB12029



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## INTENDED USE AND PRESENTATION:

This polymerase is a high fidelity enzyme widely used in molecular biology for the amplification of extremely difficult templates and long amplicons.

**AB12027, 500 units.** Concentration of enzyme 5 units/ $\mu$ L.

**AB12028, 1000 units.** Concentration of enzyme 5 units/ $\mu$ L.

**AB12029, 2500 units.** Concentration of enzyme 5 units/ $\mu$ L.

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10  $\eta$ moles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

For research use only.

## SUMMARY, EXPLANATION AND LIMITATIONS:

Nova Nova Pfu DNA Polymerase has both 5' - 3' exonuclease functions.

Therefore, it has up to 10 times less error-rate of any thermostable DNA polymerase studied, and it is efficient for cloning (High Fidelity).

Nova Pfu DNA Polymerase has been purified from the Recombinant *E. coli* strain with cloned gene encoding *Pyrococcus furiosus* DNA polymerase.

**Quality Control:** Activity and stability tested at 20, 30 and 40 cycles of PCR reactions at 95°C. Tested for the absence of human DNA contamination by PCR with Alu-specific primers.

## APPLICATIONS:

The Nova Pfu Polymerase is a robust enzyme for all your everyday PCR applications including genotyping, screening and library construction. This Pfu DNA Polymerase can perform consistently well on a broad range of templates (including both GC and AT rich).

## PRODUCT COMPOSITION:

-Storage Buffer: 20 mM Tris-HCl (pH 8,0), 1 mM DTT, 0,1 mM EDTA, 100 mM KCl, 0,5% Nonidet P40, 0,5% Tween 20 and 50% glycerol.

-10X Reaction Buffer: 200 mM Tris-HCl (pH 8,8 at 25°C), 100 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1% Triton X-100, 1 mg/ml BSA.

-10X Reaction Buffer with MgSO<sub>4</sub>: 200 mM Tris-HCl (pH 8,8 at 25°C), 100 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1% Triton X-100, 1 mg/ml BSA, 20 mM MgSO<sub>4</sub>.

-25 mM MgSO<sub>4</sub> solution.

## METHODS AND PROCEDURE:

Optimal reaction conditions, such as reaction time, temperature, and amount of template DNA, may vary and must be individually determined.

## General Reaction Protocol:

1. Thaw 10 fold concentrated reaction buffer and dNTPs mixture.
2. Prepare a master mix.

## Recommended PCR reaction mix:

Component	Volume	Final Conc.
10X Reaction Buffer (or MgSO <sub>4</sub> )	5 $\mu$ L	1X
10 mM dNTPs Mixture	1 $\mu$ L	0,2 mM
Upstream Primer	0,3-1,0 $\mu$ L	0,3-1,0 $\mu$ M
Downstream Primer	0,3-1,0 $\mu$ L	0,3-1,0 $\mu$ M
Nova Pfu DNA Polymerase (5 U/ $\mu$ L)	0,1-0,4 $\mu$ L	1,25-2,5 U
25 mM MgSO <sub>4</sub>	3-5 $\mu$ L	1,25-2,5 mM
Template DNA	Variable	1-100 ng/ $\mu$ l
Sterilized D.W.	up to 50 $\mu$ L	-
Total Volume	50 $\mu$ L	-

\*Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (<http://frodo.wi.mit.edu/primer3/>).

Amount of template:

Bacteriophage  $\lambda$ , cosmid, plasmid  $\rightarrow$  1 pg~5 ng.

Total genomic DNA  $\rightarrow$  10 ng~250 ng.

3. Mix the master mix and dispense appropriate volumes into PCR tubes. Centrifuge the reactions in a microcentrifuge for 10 seconds.
4. Perform PCR using your standard parameters (3-step).

Step	Temperature & Reaction Time		
Initial denaturation	3-5 min. at 95°C	-	-
25-35 cycles	30-60 sec. at 95°C	30-60 sec. at 50~68°C	1-4 min. at 72°C
Final extension	-	-	5-10 min at 72°C

\*For PCR products longer than 3~4 Kb, use an extension time of approximately 1 min per Kb DNA.

5. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

## REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for PCR procedures are not provided with this polymerase. This includes sterile reaction tubes, micropipettes and tips, template DNA, gen-specific PCR primer pair, PCR grade H<sub>2</sub>O, heat pretreatment equipment (thermoblock, microwave), centrifuge, cold store and thermal block cycler.

Buffered solutions for DNA extraction or purification, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

## STORAGE AND STABILITY:

Store at -20°C until the expiration date printed on product label. Avoid prolonged exposure to light. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Do not use after the expiration date. If the product is stored under different conditions from those stipulated in



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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these technical indications, the new conditions must be verified by the user. The validity period of the ready to use products when opened, is the same as the expiration date indicated on the label of intact product.

Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances Gennova Scientific is obliged to cover damages caused by use of this reagent.

#### **TROUBLESHOOTING:**

If unusual amplification is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the PCR system. If this does not solve the problem, please contact Gennova Scientific's technical support department ([techsupport@gennovalab.com](mailto:techsupport@gennovalab.com)) or your local distributor.

#### **PRECAUTIONS:**

Use only by qualified personnel.

Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific amplification results.

Material safety data sheet (MSDS) is available upon request.

#### **PERFORMANCE CHARACTERISTICS:**

Gennova Scientific has performed studies to evaluate the functioning of this polymerase for use with standard amplification systems, concluding that the product is both specific and sensitive for PCR performance.

#### **BIBLIOGRAPHY:**

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