

# Nova HotTaq DNA Polymerase

Without dNTPs

Reference: AB12036; AB12037; AB12038



1 of 2

## INTENDED USE AND PRESENTATION:

This polymerase is a hot start enzyme widely used in molecular biology.

**AB12036, 500 units.** Concentration of enzyme 10 units/ $\mu\text{L}$ .

**AB12037, 100 units.** Concentration of enzyme 10 units/ $\mu\text{L}$ .

**AB12038, 2500 units.** Concentration of enzyme 10 units/ $\mu\text{L}$ .

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

For research use only.

## SUMMARY, EXPLANATION AND LIMITATIONS:

Nova HotTaq DNA Polymerase is a chemically modified Nova Taq DNA Polymerase. At ambient temperatures it is inactive, having no polymerization activity. This HotTaq DNA Polymerase is activated by a 15 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR setup. The enzyme has 5'  $\rightarrow$  3' polymerization-dependent exonuclease replacement activity but lacks 3'  $\rightarrow$  5' exonuclease activity.

Have been purified from an *E.coli* strain that carries an overproducing plasmid containing a modified gene of *Thermus aquaticus* 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 HotTaq Polymerase is a robust enzyme for all your everyday PCR applications including genotyping, screening and library construction. This HotTaq 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), 1mM DTT, 0,1 mM EDTA, 100 mM KCl, 0,5% Nonidet P40, 0,5% Tween 20 and 50% glycerol.

-10X Reaction Buffer: 100 mM Tris-HCl (pH 8,8 at 25°C), 500 mM KCl, 0,8% Nonidet P40.

-10x Reaction Buffer with  $(\text{NH}_4)_2\text{SO}_4$ : Tris-HCl (pH 8,8 at 25°C), 200 mM  $(\text{NH}_4)_2\text{SO}_4$ , 0,1% Tween 20.

-25 mM  $\text{MgCl}_2$ .

## 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 with $(\text{NH}_4)_2\text{SO}_4$ )	5 $\mu\text{L}$	1X
10 mM dNTPs Mixture	1 $\mu\text{L}$	0,2 mM
25 mM $\text{MgCl}_2$	3-5 $\mu\text{L}$	1,5-2,5 mM
Upstream Primer (10 pmol/ $\mu\text{L}$ )	variable	0,3-1,0 $\mu\text{M}$
Downstream Primer (10 pmol/ $\mu\text{L}$ )	variable	0,3-1,0 $\mu\text{M}$
Nova HotTaq DNA Polymerase (10 U/ $\mu\text{L}$ )	variable	1,25-2,5 U
Template DNA	variable	1-100 $\eta\text{g}/\mu\text{L}$
Sterilized D.W.	Up to 50 $\mu\text{L}$	-
Total Volume	50 $\mu\text{L}$	-

\*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  $\eta\text{g}$ .

Total genomic DNA  $\rightarrow$  10  $\eta\text{g}$ -250  $\eta\text{g}$ .

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	15 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

\*Annealing temperature should be 2-6°C lower than the primer melting temperature.

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

A DNA fragment which is amplified by Nova Taq DNA Polymerase has A-overhang, and it enables you to do cloning by using T-vector.

## 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  $\text{H}_2\text{O}$ , 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.



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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2 of 2

### 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 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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