EcoNova HotTaq DNA Polymerase

Without dNTPs

Reference: AB12024; AB12025; AB12026

INTENDED USE AND PRESENTATION:

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

AB12024, 500 units. Concentration of enzyme 5 units/µL.
AB12025, 1000 units. Concentration of enzyme 5 units/µL.
AB12026, 2500 units. Concentration of enzyme 5 units/µL.
One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 ηmoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.
For research use only.

SUMMARY, EXPLANATION AND LIMITATIONS:

EcoNova HotTaq DNA Polymerase is a chemically modified EcoNova 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'→ 3' polymerization-dependent exonuclease replacement activity but lacks 3'→ 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: The enzyme is free of nicking and priming activities, exonucleases and non-specific endonucleases. SDS PAGE -95 kD band, >98% pure. Activity and stability tested via thermo cycling. Has an error rate of approximately 1 error per $2,5x10^5$ nucleotides incorporated; the accuracy is approximately $4x10^4$. Estimated half-life at 95ºC is 1,5 hours.

APPLICATIONS:

The EcoNova 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: 50% glycerol (v/v), 20 mM Tris-HCl pH 8,7 at 25ºC, 100 mM KCl, 0,1 mM EDTA and stabilizers.
-10X Reaction Buffer B1 (Mg^{2+}, detergent free): Tris-HCl and $(NH_4)_2SO_4$.
-10x Reaction Buffer B2 (Mg^{2+} free): Tris-HCl, $(NH_4)_2SO_4$ and detergent.
-25 mM $MgCl_2$.
-10X Enhancer: Additive that facilitates amplification of difficult templates (e.g. GC-rich DNA templates). Enhancer should be used at a defined working concentration (1x, 2x or 3x solution).

Enhancer is NOT a reaction buffer and should be used ONLY IF non-specific amplifications occur.

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 B1 or B2	10 µL	1X
20 mM dNTPs Mixture	1 µL	0,2-1 mM
25 mM $MgCl_2$	6-10 µL	1,5-2,5 mM
Upstream Primer (10 pmol/µL)	1-3 µL	0,1-0,3 µM
Downstream Primer (10 pmol/µL)	1-3 µL	0,1-0,3 µM
EcoNova HotTaq DNA Polymerase (5 U/µL)	0,4-1,0 µL	2,0-5,0 unit
Template DNA	5-20 µL	5-100 ηg/µL
10X enhancer **Not for standard PCR**	0, 10, 20, or 30 µL	1x, 2x or 3x
Sterilized D.W.	Up to 100 µL	-
Total Volume	100 µL	-

*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 λ, cosmid, plasmid → 1 pg-5 ηg.
Total genomic DNA → 10 ηg-250 ηg.

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	12-15 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

***IMPORTANT:** To activate the polymerase, include an incubation step at **95ºC for 12 - 15 minutes** at the beginning of the PCR cycle. Annealing temperature should be 2-6ºC lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

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 coning by using T-vector.

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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_2O, 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 Gennova 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 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) 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.

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Catalog number

Batch code

Research use only

Temperature limitation

Expiration date

Manufacturer

See instruction for use

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