Nova LongTaq DNA Polymerase

Without dNTPs

Reference: AB12018; AB12019; AB12020

INTENDED USE AND PRESENTATION:

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

AB12018, 500 units. Concentration of enzyme 5 units/µL.

AB12019, 1000 units. Concentration of enzyme 5 units/µL.

AB12020, 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:

Nova LongTaq DNA Polymerase is the mixture of two DNA polymerases designed for long PCR. The mixture allows to amplify genomic DNA fragments up to 20 kb and viral DNA fragments up to 40 kb.

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 LongTaq Polymerase is a robust enzyme for all your everyday PCR applications including genotyping, screening and library construction. This Taq 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), 100 mM KCl, 0,1 mM EDTA, 1 mM DTT, 0,5% Tween 20, 0,5 % Nonidet P-40, 50% Glycerol.

-10X Reaction Buffer: Contains 100 mM Tris-HCl (pH 8,8), PCR enhancers, $(NH_4)_2SO_4$, 500 mM KCl.

-25 mM $MgCl_2$.

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 (not provided).

2. Prepare a master mix.

Recommended PCR reaction mix:

Component	Volume	Final Conc.
10X Reaction Buffer	5 µL	1X
10 mM dNTPs Mixture	1-5 µL	0,2-1 mM
Upstream Primer	Variable	0,3-1,0 µM
Downstream Primer	Variable	0,3-1,0 µM
Nova LongTaq DNA Polymerase (5 U/µL)	0,2-0,5 µL	1,25-2,5 unit
Template DNA	Variable	1-100 ηg/µL
Sterilized D.W.	Up to 50 µL	-
Total Volume	50 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 → 10 fg-300 ηg.
Total genomic DNA → 100 ηg-1 µg.

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).

PCR cycles for <10 kb fragments:

Step	Temperature & Reaction Time		
Initial denaturation	2-4 min. at 92-94°C	-	-
25-30 cycles	15 sec-1 min. at 94°C	15 sec-1 min. at 45-65°C	1 min. per 1-1,5 kb*
Final extension	-	-	5-10 min at 72°C

* Extension time for 30s-1min is sufficient for fragments < 1kb

PCR cycles for >10 kb fragments:

Step	Temperature & Reaction Time		
Initial denaturation	2-4 min. at 92-94°C	-	-
10 cycles	5 sec-1 min. at 94°C	15 sec-1 min. at 45-65°C	1 min. per 1-1,5 kb
15-20 cycles	5 sec-1 min. at 94°C	15 sec-1 min. at 45-65°C	1 min. per 1-1,5 kb + 20 sec/cycle
Final extension	-	-	5-10 min. at 72°C

Annealing temperature and time need to be optimized for each primer/template combination.

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.

Gennova Scientific, S.L.
C/ Johann Gutenberg, 4F. Pol. Ind.
El Cáñamo I • 41300 San José
de La Rinconada • Sevilla, SPAIN
Teléfono: +34 954 150767
Fax: +34 955 266494
info@gennovalab.com
www.gennova-europe.com

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, dNTPs mixture, 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.

BIBLIOGRAPHY:

Chien A., Edgar D.B., Trela J.M., "Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*", Journal of Bacteriology, 127(3), 1550-57, 1976.
Lawyer F.C., Stoffel S., Saiki R.K., Myambo K., Drummond R., et al., "Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from Thermus aquaticus", The Journal of Biological Chemistry, 264(11), 6427-37, 1989.
Tindall K.R., Kunkel T.A., "Fidelity of DNA synthesis by the *Thermus aquaticus* DNA polymerase", Biochemistry, 27(16), 6008-13, 1988.
Innis M.A., Myambo K.B., Gelfand D.H., Brow M.A., "DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA", Proceedings of the National Academy of Sciences of the United States of America, 85(24), 9436-40, 1988.
Lo Y.M., Mehal W.Z., Fleming K.A., "Rapid production of vector-free biotinylated probes using the polymerase chain reaction", Nucleic Acids Research, 16(17), 8719, 1988.
Erlich H.A., (ed.) 1988, "PCR technology: principles and applications for DNA amplification", Stockton Press, New York.

REF Catalog number | LOT Batch code | RUO Research use only
Temperature limitation | Expiration date
Manufacturer | See instruction for use

Gennova Scientific, S.L.
C/ Johann Gutenberg, 4F. Pol. Ind.
El Cáñamo I • 41300 San José
de La Rinconada • Sevilla, SPAIN
Teléfono: +34 954 150767
Fax: +34 955 266494
info@gennovalab.com
www.gennova-europe.com