LongNova DNA Polymerase

Components	RP281 200 U	RP282 1000 U
***LongNova* 2 U/µl** DNA Polymerase	100 µl	500 µl
10x *LongNova 1* Reaction Buffer	2 ml	5 x 2 ml
10x *LongNova 2* Reaction Buffer	2 ml	5 x 2 ml
50 mM $MgCl_2$	1 ml	4 x 1 ml

Storage & shipping

Storage conditions
Store all components at -20°C.

Shipping conditions
Shipping on dry or blue ice.

For research use only

Date of purchase

Warranty
12 months from the date of purchase

BLIRT S.A. DNA-Gdansk Division
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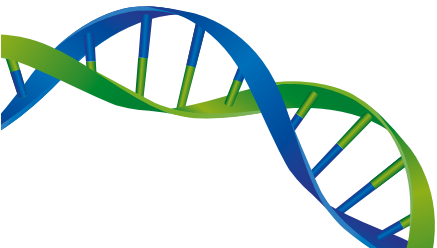
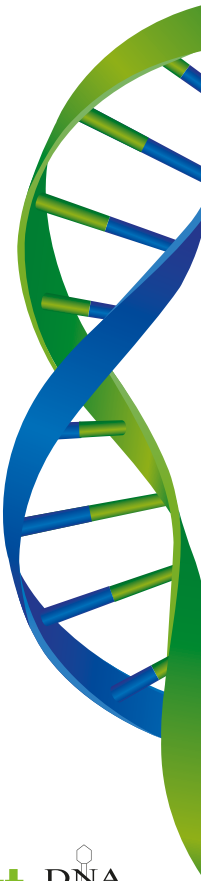
LongNova

DNA Polymerase





blirt® DNA GDAŃSK

LongNova
DNA Polymerase

LongNova DNA Polymerase is a mixture of thermostable *Pwo* and *Taq* polymerases. It catalyses DNA synthesis in a 5'→ 3' direction and shows a 3'→ 5' exonuclease activity. The thermostable ***LongNova*** DNA Polymerase is characterized by high processivity and proofreading properties. It is ideal for long range PCR (up to 20 kb) and GC-rich template amplification applications.

Features and advantages

- → Increased processivity (for very long amplicons)
- → High yield with minimal amounts of enzyme and little optimization (routine PCR)
- → Wide range of product sizes – from 2 to 20 kb
- → High proofreading properties (3'→5' exonuclease activity)
- → Leaves ´A´ overhangs

Applications

- → Very long PCR products
- → Molecular cloning
- → Site-directed mutagenesis and other methods which require high fidelity

blirt DNA GDAŃSK

LongNova
DNA Polymerase

Protocol

1. Prior to use, thaw the reagents completely, mix thoroughly and spin briefly.
2. Add the following reaction reagents to a sterile nuclease-free PCR Eppendorf tube:

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
10x *LongNova 1 or 2*	5 µl	1x
8 mM dNTPs Mix	5 µl	0.2 – 0.25 mM of each dNTP
50 mM $MgCl_2$	2 µl	2 – 5 mM
10 µM Forward primer	1 µl	0.1 – 1.0 µM
10 µM Reverse primer	1 µl	0.1 – 1.0 µM
DNA template	1 – 100 ng	10 pg – 0.5 µg
***LongNova* DNA Polymerase**	**1 U**	**1 – 2 U**
PCR – grade water	fill up to 50 µl	fill up to required volume

This composition is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimising.

3. Mix the prepared reaction mixture thoroughly by pipetting or vortexing, then spin briefly.

4. Place the prepared PCR mixture in a thermal cycler and start the PCR reaction. The table below shows a sample PCR cycling conditions.

Step	Temperature [°C]	Time	
Initial denaturation	95°C	1–5 min [1]	
Denaturation	95°C	15 s	30-40 cycles [4]
Annealing	45–65°C [2]	30 s	
Extension	68°C	2–20 min [3]	
Final extension	72°C	5 min	
Cooling	4°C	∞	

1) The initial denaturation time depends on the GC content within the amplified region and the template DNA type. For non-complex templates, such as plasmid DNA or cDNA, the initial denaturation step, carried out briefly (1–2 min), is recommended. For more complex templates, such as eukaryotic genomic DNA, a longer initial denaturation step (3–5 min) is required.
2) The annealing temperature depends on the primer sequences and their melting temperature (Tm). The optimal annealing temperature is usually 2–5°C below the Tm of primers.
3) The elongation time depends on the length of an amplified product. Setting 1 min per 1000 bp of the amplified product is recommended.
4) The number of cycles depends on the number of copies of the amplified gene fragment. Thirty cycles is sufficient for low complexity templates. In the case of high complexity templates or less concentrated DNA, the number of cycles should be increased to forty.

5. After reaction has finished, apply the reaction mixtures onto a gel.

Troubleshooting

For problems which may be encountered during PCR reaction set up and analysis, possible causes and solutions see: www.dnagdansk.com.

Storage buffer

20 mM Tris-HCl (pH 8.0, 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20, 50% (v/v) glycerol

Quality control

Free of unspecific nucleases and DNA contamination. The amplification and proofreading activities of the polymerase are tested in appropriate assays. Extensively tested in various long range PCR reactions.

Unit definition

One unit is defined as an amount of enzyme required to incorporate 10 nmol of dNTPs to an insoluble DNA fraction in 30 minutes at 75°C in a 50 µl reaction.