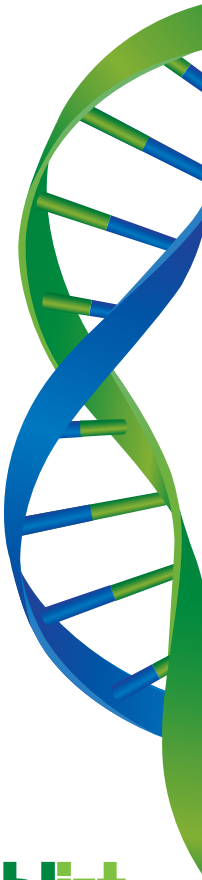
TaqNova

DNA-free Polymerase



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DNA-free Polymerase

***TaqNova* DNA-free** Polymerase is a 94 kDa recombinant, thermostable *Taq* DNA polymerase isolated from *Thermus aquaticus*. It is recommended for a wide range of applications which require DNA synthesis at extremely high temperatures. The ***TaqNova* DNA-free** Polymerase is a universal and easy-to-use DNA polymerase which works rapidly and effectively in various PCR condition. The enzyme catalyzes DNA synthesis in a 5'→ 3' direction, shows no 3'→ 5' exonuclease activity, but has a 5'→ 3' exonuclease activity.

***TaqNova* DNA-free** Polymerase is highly purified from DNA contaminants, enabling amplification of very conserved sequences (e.g. bacterial 16S rRNA region) without risk of false positive PCR results.

Features and advantages

- → Extreme yield with minimal amounts of enzyme and little optimization
- → Increased sensitivity
- → Suitable for a wide range of applications
- → Consistent results
- → High-purity recombinant enzyme from DNA contaminants
- → The half-life of the enzyme is 45 minutes at 95°C
- → Amplifies fragments of up to 5 kb
- → Leaves ´A´ overhangs

Applications

- → Efficient amplification of conserved sequences (e.g. bacterial 16S rRNA region)
- → Routine PCR
- → Diagnostic PCR
- → Multiplex PCR
- → TA cloning

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Protocol

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

Reagent	Suggested amount per reaction	Acceptable final concentrations in a reaction mixture
10x *TaqNova* DNA-free Reaction Buffer	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
***TaqNova* DNA-free Polymerase**	**1 U**	**1 – 2 U**
PCR-grade water	fill up to 50 µl	

This composition is intended for use as a guide only; conditions may vary from reaction to reaction and may require optimization.

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 the suggested PCR cycling conditions.

Step	Temperature	Time	
Initial denaturation	94–95°C	1–5 min [1]	
Denaturation	94–95°C	30 s	25–40 cycles [4]
Annealing	45–65°C [2]	30 s	
Extension	72°C	15 s–2 min [3]	
Final extension	72°C	1–5 min	
Cooling	4°C	∞	

1) The initial denaturation time depends on the GC content within the amplified region and template DNA type. For non-complex templates, such as plasmid DNA or cDNA, the initial denaturation step of 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 DNA fragment. Setting 30 seconds per 1 kbp of the PCR product is recommended.
4) The number of cycles depends on the number of copies of the amplified gene fragment. Thirty cycles are sufficient for low complexity templates. In case of high complexity templates or less concentrated DNA, the number of cycles should be increased to forty.

Troubleshooting

For solutions to problems that may occure during PCR set up or analysis, please search in FAQ section at www.blirt.eu or contact support@blirt.eu.

Storage buffer

20 mM Tris-HCl (pH 8.0 at 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.

10 x *TaqNova* DNA-free Reaction Buffer

100 mM Tris-HCl (pH 8.3 at 25°C), 500 mM KCl

Quality control

- → Purity > 95% determined by densitometry of SDS-PAGE
- → Functionality of the enzyme tested in PCR reaction with human DNA
- → Free of nonspecific DNase contamination
- → DNA contamination is evaluated by qPCR reaction with primers specific for 16S rDNA region, less than 1 *E. coli* genome is detected in 1 U of enzyme

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 72°C in a 50 µl reaction.

TaqNova **DNA-free** Polymerase

Components	RP1002 200 U	RP1010 1 000 U	RP1002-S 20 U
	***TaqNova* DNA-free Polymerase 5 U/µl**		
***TaqNova* DNA-free** Polymerase **5 U/µl**	40 µl	200 µl	4 µl
10x *TaqNova* DNA-free Reaction Buffer	1.25 ml	4x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	4x 1 ml	80 µl

Component	RP1000HC
***TaqNova* DNA-free** Polymerase **(100 U/µl)**	Upon request

Storage & shipping

Storage conditions
Store all components at -20°C.

Shipping conditions
Shipping on dry or blue ice.

For research use only

Expiry
Information on the label.

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