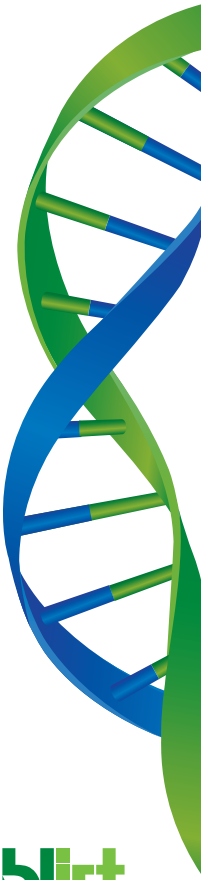
TaqNovaGC

DNA Polymerase



blirt

1.2017

TaqNovaGC

DNA Polymerase

TaqNovaGC DNA Polymerase is an ideal tool for the amplification of GC-rich templates. A recombinant and thermostable enzyme isolated from *Thermus aquaticus*, is recommended for a wide range of applications requiring DNA synthesis at extremely high temperatures. The ***TaqNovaGC*** DNA polymerase is a universal DNA polymerase which works rapidly and effectively in various PCR conditions. The enzyme catalyses DNA synthesis in a 5'→ 3' direction, shows no 3'→ 5' exonuclease activity, but has a 5'→3' exonuclease activity.

The application of appropriate PCR buffer conditions and **5x *GC-Additive*** as the PCR enhancer enables the amplification of specific DNA regions with a high GC-content. This PCR additive changes DNA behavior upon heating and can be used with GC-rich primer-template pairs which do not work well under standard PCR conditions. The **5x *GC-Additive*** reduces the number of secondary structures and enables the specific hybridisation of primers.

Features and advantages

- → Recombinant enzyme of high purity
- → Extreme yield with minimal amounts of enzyme and little optimization
- → Half-life of the enzyme is 45 minutes at 95°C
- → Amplifies fragments of up to 5 kb
- → High specificity
- → Enables the amplification of specific DNA regions with a high GC-content

Applications

- → Efficient amplification of short and medium size DNA sequences
- → GC-rich templates
- → Ideal for problematic templates, which fail with standard *Taq* DNA polymerases
- → Diagnostic PCR
- → TA cloning

TaqNovaGC
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 *TaqNovaGC* 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
5x *GC-Additive*	10 µl	1-2x
10 µM Forward primer	1 µl	0.2–1.0 µM
10 µM Reverse primer	1 µl	0.2–1.0 µM
DNA template	1–100 ng	10 pg–0.5 µg
***TaqNovaGC* DNA Polymerase**	**1 U**	**1 – 2 U**
PCR – grade water	fill up to 50 µl	fill up to required volume

* **5x *GC-Additive*** should be used at 1.0-2.0x final concentration – the optimal amount required should be determined for each individual experiment

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 a sample PCR cycling conditions.

Step	Temperature	Time	
Initial denaturation	95°C	1–5 min [1]	
Denaturation	95°C	30 s	30-40 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 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 20–30 s 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 reaction mixtures onto a gel.

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

Troubleshooting

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

Quality control

Free of nonspecific nucleases (DNases) contamination. Extensively tested in PCR applications (GC rich regions).

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.

TaqNovaGC DNA Polymerase

Components	RP73-020 200 U	RP73-100 1000 U	RP73-S 20 U
***TaqNovaGC* 5 U/µl** DNA Polymerase	40 µl	200 µl	4 µl
10x *TaqNovaGC* Reaction Buffer	1.25 ml	4x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	4x 1 ml	80 µl
5x *GC-Additive*	1 ml	5x 1 ml	100 µl

Storage & shipping

Storage conditions
Store all components at -20°C.

Shipping conditions
Shipping on dry or blue ice.

For research use only

Expiry
The information on the label

BLIRT S.A.
orders@blirt.eu | www.blirt.eu