TaqNova

DNA Polymerase

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DNA Polymerase

TaqNova DNA 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 polymerase is a universal and easy-to-use 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.

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
- → 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 short and medium size DNA sequences
- → 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 Eppendorf tube:

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
10x *TaqNova* 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 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 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 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 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 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.

Additional information

Both reaction buffers provided may be used with ***TaqNova*** DNA polymerase. **10 x *TaqNova* KCl** buffer is recommended as first approach and for applications requiring high specificity. **10 x *TaqNova*** $(NH_4)_2SO_4$ buffer is recommended for applications where high sensitivity and amplification efficiency is required (e.g. for amplification of multiple DNA fragments).

Both buffers may be evaluated to determine the buffer most suitable for specific application.

Troubleshooting

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

Reaction buffers

10 x *TaqNova* KCl
100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% (v/v) Nonidet P40

10 x *TaqNova* $(NH_4)_2SO_4$
750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween 20

Quality control

Free of nonspecific nucleases (DNases) contamination. Extensively tested in 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 72°C in a 50 µl reaction.

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Components	RP702A 200 U	RP705A 500 U	RP710A 1000 U	RP725A 2500 U	RP702A-S 20 U
			***TaqNova* DNA Polymerase 5 U/µl**		
***TaqNova* 5 U/µl** DNA Polymerase	40 µl	100 µl	200 µl	500 µl	4 µl
10x *TaqNova* KCl Reaction Buffer	1.25 ml	2 x 1.25 ml	4 x 1.25 ml	10 x 1.25 ml	100 µl
10x *TaqNova* $(NH_4)_2SO_4$ Reaction Buffer	1.25 ml	2 x 1.25 ml	4 x 1.25 ml	10 x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	2 x 1 ml	4 x 1 ml	10 x 1 ml	80 µl

Components	RP702 200 U	RP705 500 U	RP710 1000 U	RP725 2500 U	RP702-S 20 U
			***TaqNova* DNA Polymerase 2 U/µl**		
***TaqNova* 2 U/µl** DNA Polymerase	100 µl	250 µl	500 µl	1250 µl	10 µl
10x *TaqNova* KCl Reaction Buffer	1.25 ml	2 x 1.25 ml	4 x 1.25 ml	10 x 1.25 ml	100 µl
10x *TaqNova* $(NH_4)_2SO_4$ Reaction Buffer	1.25 ml	2 x 1.25 ml	4 x 1.25 ml	10 x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	2 x 1 ml	4 x 1 ml	10 x 1 ml	80 µl

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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