TaqNova-RED

DNA Polymerase

1.2017

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DNA Polymerase

TaqNova-RED DNA Polymerase is a 94 kDa, recombinant, thermostable *Taq* DNA polymerase isolated from *Thermus aquaticus* with an addition of inert, red dye. It facilitates accurate low volume pipetting and is an indicator of an enzyme addition. The dye does not affect the outcome of PCR adversely; the efficiency is identical as with the standard ***TaqNova*** DNA Polymerase.

The thermostable ***TaqNova-RED*** DNA Polymerase catalyzes DNA synthesis in 5'→ 3' direction, shows no 3'→ 5' exonuclease activity, but demonstrates 5'→ 3' exonuclease activity.

Use of the ***TaqNova-RED*** DNA Polymerase decreases the risk of error occurrence during preparation of reactions e.g. omission of the polymerase or mixing inaccurate reagents. PCR product may be directly applied to a gel after amplification process is completed, with no need of mixing with loading buffer. It is less time consuming and facilitates a preparation of agarose or polyacrylamide gel electrophoresis. A fresh TAE/TBE buffer is recommended to use for the agarose gel preparation and electrophoretic processes.

The dye does not interfere with any successive reactions (except reactions employing fluorescence techniques) and PCR products may be easily separated from the dye by any standard DNA purification method (e.g. PCR/DNA Clean-up Purification Kit).

Features and advantages

- → Consistent results
- → Suitable for a wide range of applications
- → 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
- → Leaves ´A´ overhangs
- → Facilitate PCR reaction set-up
- → Direct gel loading

Applications

- → Efficient amplification of short and medium size DNA sequences
- → Routine PCR
- → Diagnostic PCR
- → TA cloning

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Protocol

Use of the ***TaqNova-RED*** DNA Polymerase does not require any changes in the reaction set-up or additional procedures:

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 *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 ng	10 pg–0.5 µg
***TaqNova-RED* DNA Polymerase 1 U/µl**	**2 µl**	**1 – 3 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 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 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. For 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-RED*** DNA polymerase. **10x *TaqNova* KCl** buffer is recommended as first approach and for applications requiring high specificity. **10x *TaqNova* $(NH_4)_2SO_4$** buffer is recommended for applications where high amplification efficiency is required (e.g. for multiple products' amplification). Both buffers may be evaluated to determine the one that is most suitable for specific application.
- The recommended concentration of ***TaqNova-RED*** DNA Polymerase in the reaction is 0.04 U/µl. If other polymerase concentrations are needed, magnesium ion concentration optimization may be required. The table below presents Mg^{2+} concentration calculations for the volume of 50 mM $MgCl_2$ added to the reaction.

Final concentration of Mg^{2+} [mM]	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
	Reaction volume 50 µl						
50 mM $MgCl_2$ [µl]	1.0	1.5	2.0	2.5	3.0	3.5	4.0

- A minimal volume of the polymerase in a reaction is 1.5 µl / 50 µl, for the efficient sample loading to a gel.
- PCR products may be applied directly onto a gel in a volume range of 5–15 µl with no need to mix them with a DNA loading dye. Electrophoresis, detection and visualization may be carried out in accordance with the standard procedures.

Troubleshooting

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

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, inert dye and additives, 50% (v/v) glycerol

Reaction buffers

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

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

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.

Quality control

Free of unspecific DNA nucleases.
Functionality tested in PCR reaction.

TaqNova-RED DNA Polymerase

Components	RP20R 200 U	RP100R 1000 U	RP20R-S 20 U
***TaqNova-RED* 1 U/µl** DNA Polymerase	200 µl	1000 µl	20 µl
10x *TaqNova* KCl Reaction Buffer	1.25 ml	4x 1.25 ml	100 µl
10x *TaqNova* $(NH_4)_2SO_4$ Reaction Buffer	1.25 ml	4x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	4x 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
The information on the label

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