August 2023

Quick-Start Protocol

TaqNova DNA Polymerase

TaqNova DNA Polymerase (cat. nos. RP702A, RP725A, and RP705A) is a 94 kDA recombinant, thermostable Taq DNA polymerase isolated from *Thermus aquaticus*. It is recommended for a wide range of applications that require DNA synthesis at extremely high temperatures. TaqNova DNA Polymerase is a universal and easy-to-use DNA polymerase that works rapidly and effectively in various PCR conditions. The enzyme catalyzes DNA synthesis in 5′ → 3′ direction, shows no 3′ → 5′ exonuclease activity, but has a 5′ → 3′ exonuclease activity. TaqNova DNA Polymerase must be shipped on dry ice, and stored at –20°C.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- 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.

Things to do before starting

1. 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 reaction mixture
10x 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 to 0.5 µg
TaqNova DNA Polymerase	1 U	1–2 U
PCR-grade water	Fill up to 50 µL	

*Both reaction buffers provided may be used with TaqNova DNA Polymerase. 10x TaqNova KCl Buffer is recommended as the first approach for applications requiring high specificity. 10x TaqNova (NH4,) 2SO2 buffer is recommended for applications where high sensitivity and amplification efficiency are required (e.g. for amplification of multiple DNA fragments). Both buffers may be evaluated to determine the buffer most suitable for a specific application.

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

Procedure

1. Mix the prepared reaction mixture thoroughly by pipetting or vortexing, then spin briefly.
2. Place the prepared PCR mixture in a thermal cycler and start the PCR reaction. Table 1 shows the suggested PCR cycling conditions.

Table 1. Suggested PCR cycling conditions

Step	Temperature (°C)	Time	
Initial denaturation	94–95	1–5 min*	
Denaturation	94–95	30 s	
Annealing	45–65 †	30 s	25–40 cycles§
Extension	72	15 s to 2 min‡	
Final extension	72	1–5 min	
Cooling	4	∞	

* 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.

† 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.

‡ The elongation time depends on the length of an amplified DNA fragment. Setting 30 s per 1 kbp of the PCR product is recommended.

§ 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 40.

Document Revision History

Date	Changes
08/2023	Initial release

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