

Quick-Start Protocol

Stoffel DNA Polymerase

Stoffel DNA Polymerase (cat. no. RP810) is a 62.7 kDa recombinant, fragment of thermostable Taq DNA polymerase isolated from *Thermus aquaticus*. It is recommended for a wide range of applications, which require DNA synthesis in extremely high temperatures. Stoffel DNA Polymerase is an universal and easy-to-use DNA polymerase that works rapidly and effectively in various PCR conditions. The enzyme catalyses DNA synthesis in a 5' → 3' directions, it does not show a 3' → 5' and 5' → 3' exonuclease activity. Stoffel 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 min 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 Stoffel buffer	5 µL	1x
8 mM dNTPs Mix	5 µL	0.2–0.25 mM of each dNTP
50 mM MgCl ₂	3 µL	2–10 mM (optimum 3–5 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
Stoffel DNA Polymerase	1 U	0.5–2 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.

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 (next page) shows suggested PCR cycling conditions.

Table 1. Suggested PCR cycling conditions

Step	Temperature (°C)	Time	No. of cycles
Initial denaturation	94–98	1–5 min*	–
Denaturation	94–98	30 s	25–40 cycles [§]
Annealing	45–65 [†]	30 s	
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 DNA template type. For non-complex templates, such as plasmid DNA or cDNA, the 1–2 min initial denaturation step is recommended. For more complex templates, such as eukaryotic genomic DNA, a higher temperature (98°C) is recommended and longer initial denaturation step (3–5 min) is required.

[†] The annealing temperature depends on the primer sequences and their melting temperature (*T*_m). The optimal annealing temperature is usually 2–5°C below the *T*_m of primers.

[‡] The elongation time depends on the length of an amplified DNA fragments, 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
07/2024	Revision of Trademarks section

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