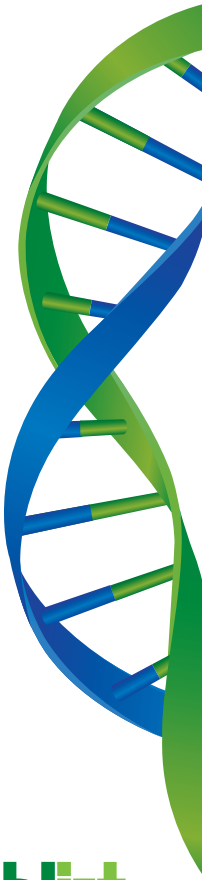
TaqNova Stoffel

DNA Polymerase



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DNA Polymerase

TaqNova Stoffel DNA Polymerase 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. ***TaqNova 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 fragment is encoded by a modified form of the *Thermus aquaticus* DNA polymerase gene which has been inserted into an *Escherichia coli* host. The modified gene encodes a 540 amino acid fragment lacking the N-terminal 292 amino acid portion of the full length TaqNova DNA Polymerase.

The thermostability of *TaqNova Stoffel* DNA Polymerase is about twice as high as the *TaqNova* DNA Polymerase and requires higher $MgCl_2$ concentration level and lower ionic strenght for its optimum enzymatic activity.

Features and advantages

- → Consistent results
- → Suitable for a wide range of applications
- → High-purity recombinant enzyme – confirmed 95% purity recombinant enzyme
- → High efficiency enzyme – extreme yields

- → Easy to use – no optimization required
- → Maximum performance with improved reaction buffer formulation
- → No exonuclease activity
- → High thermostability – half-life of the enzyme is 20 minutes at 97.5°C
- → Amplifies fragments of up to 5 kb
- → Leaves ´A´ overhangs

Applications

- → Efficient amplification of short and medium size sequences.
- → Diagnostic PCR.
- → *TaqNova Stoffel* DNA Polymerase is strongly sugested for GC rich and secondary structure templates – the increase thermal stability of the *TaqNova Stoffel* DNA Polymerase may lead to superior amplification of excessively GC rich templates and templates with secondary structure by allowing the use of denaturation temperatures as high as 98°C.
- → Multiplex PCR – no need for $MgCl_2$ optimisation – the vast magnesium optimum for *TaqNova Stoffel* DNA Polymerase reduces the need for magnesium optimization experiments and increases the easiness of "Multiplex PCR" optimization, the simultaneous amplification of multi targets in the same reaction.
- → Genotyping – *TaqNova Stoffel* DNA Polymerase shows great performance in genetic mapping using primers with arbitrary sequences (RAPD).
- → ASA PCR (Allele Specific Amplification PCR) – amplification depends of 3' terminal bases, that complement the primer.

NOTE: Some applications in which this product may be used may be covered by patents or patent applications applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a license depending upon the particular application and country in which the product is used.

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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 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–0.5 µg
***TaqNova 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.

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	94–98°C	1–5 min [1]	
Denaturation	94–98°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 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 temperture (98°C) is recomended and 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 fragments, 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. 30 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.

Additional information

The activity of ***TaqNova Stoffel*** DNA Polymerase is optimal at low ionic strength, thus **10x *Stoffel Buffer*** is optimized and recommended reaction buffer for this enzyme. The use of a different reaction buffer may significantly reduces the enzyme activity.

TaqNova Stoffel DNA Polymerase has a broad $MgCl_2$ optimum range (2.5–5 mM) and generally requires higher concentrations of magnesium ions than *TaqNova* DNA Polymerase. A 3 mM $MgCl_2$ concentration is a suggested starting point for PCR protocol optimization.

Troubleshooting

For solutions to problems that may occure during PCR set up or analysis, please search in FAQ section at www.blirt.eu or contact support@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 buffer

10x *Stoffel Buffer*
100 mM Tris-HCl (pH 8.3 at 25°C), 100 mM KCl

Quality control

Free of nonspecific nucleases (DNases) contamination. Extensively tested in PCR reactions.
TaqNova Stoffel DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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.

TaqNova Stoffel DNA Polymerase

Contents	1000 U (RP810)
***TaqNova Stoffel* 2 U/µl** DNA Polymerase	500 µl
10x *Stoffel Buffer* Reaction Buffer	4 x 1.25 ml
50 mM $MgCl_2$	4 x 1 ml

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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