Speedy NZYTaq DNA polymerase

Catalogue number: MB40301, 125 U
MB40302, 500 U

Description

Speedy NZYTaq DNA polymerase belongs to a new generation of DNA polymerases displaying a faster polymerization rate than any other conventional non-proofreading enzyme. Speedy NZYTaq DNA polymerase is useful for fast, routine DNA amplifications or genotyping. Only 5 seconds are required for the successful synthesis of a 1 kb size DNA fragment. The enzyme retains its speed (5 sec/kb) when amplifying fragments up to around 2-3 kb. Successful amplification of higher DNA fragments up to 6 kb in size can be reached using a 10 sec/kb extension step. Faster PCR can be further achieved by increasing the primers melting temperature, which increases PCR annealing temperature, thus allowing combining the annealing and extension PCR steps (see below). Speedy NZYTaq DNA polymerase is supplied with an optimized 10× Reaction Buffer and a 50 mM $MgCl_2$ solution. The enzyme lacks 3´→5´ exonuclease activity and supports robust and reliable reactions while tolerating a wide range of templates. PCR products generated by Speedy NZYTaq DNA polymerase have an A-overhang and are suitable for cloning with NZYTech´s TA PCR cloning kits (MB053 or MB137).

Storage Conditions

Speedy NZYTaq DNA polymerase should be stored at -20 °C in a constant temperature freezer. Speedy NZYTaq DNA polymerase will remain stable till the expiry date if stored as specified.

Unit definition

One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 72 °C.

Enzyme concentration 5 U/µL

Magnesium Chloride solution

The provided 50 mM $MgCl_2$ solution allows users to optimize $MgCl_2$ concentrations in different PCR set ups. In general, Speedy NZYTaq DNA polymerase works effectively with a 2.5 mM $MgCl_2$ concentration. Vortex the $MgCl_2$ solution thoroughly after thawing.

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (e.g. concentration of DNA polymerase, primers, $MgCl_2$, and template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune PCR conditions, recommended variations of each PCR component are provided in brackets in the table below.

1. Gently mix and briefly centrifuge all components after thawing. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of PCR reactions. Add water first and the remaining components in the order specified in the table below. A single reaction mixture of 50 µL should combine the following components:

Reaction buffer, 10× (provided)	5 µL
$MgCl_2$, 50 mM (provided)	2.5 (1.5-4.0) mM
dNTPs mix	0.4 (0.25-0.5) mM
Primers (see below)	0.25 (0.1-0.5) µM
Template DNA (see below)	50 pg-0.5 µg
Speedy NZYTaq DNA polymerase	1 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	95 °C	1 min	95 °C	1 min	1
Denaturation	94 °C	2 s	94 °C	2 s	25-35
Annealing	-	-	**(*)**	5 s	
Extension	68-72 °C **(¥)**	5 s/kb **(†)**	72 °C	5 s/kb	
Final Extension	72 °C	2 min	72 °C	2 min	1

(*) Annealing temperature should be optimized for each primer set based on the primer T_m; typically it should be T_m-5 °C.

(¥) Extension temperature will depends on primer melting temperature (see below).

(†) For DNA fragments higher than 2-3 kb to 6 kb in size, it may be beneficial to use 10 s/kb.

4. Analyse PCR products by agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with GreenSafe Premium (MB132) or any other means.

Primer Design

PCR primers generally range in length from 20–40 bases and are designed to flank the region of interest. Primers should contain 40–60% GC, and care should be taken to avoid sequences that might produce internal secondary structure. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers. Primer-dimers unnecessarily remove primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3´-end of the primer, as this may result in non-specific primer annealing. Ideally, both primers should have nearly identical melting temperatures (T_m), allowing their annealing with the denatured template DNA at roughly the same temperature. For faster PCR protocols, primers should have a $T_m \geq 60$ °C. Please note that primer annealing and product extension can be combined into one step for a faster PCR reaction (see 2-step protocol above) if primers are designed to have a $T_m \geq 70$ °C.

DNA template

The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 20ng to 500ng of genomic DNA templates, although the enzyme is

sensitive enough to amplify fragments from as little as 50pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 5-20ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity

Speedy NZYTaq DNA polymerase purity is >90% as judged by SDS-PAGE followed by Coomassie Blue staining.

Genomic DNA contamination

Speedy NZYTaq DNA polymerase must be free of any detectable genomic DNA contamination as evaluated through PCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of Speedy NZYTaq DNA polymerase, in 1× reaction buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with Speedy NZYTaq DNA polymerase buffer and $MgCl_2$ solution.

Functional assay

Speedy NZYTaq DNA polymerase is tested for performance in a polymerase chain reaction (PCR) using 5 U of enzyme for the amplification of a 1000 bp fragment from human genomic DNA. The resulting PCR product is visualized as a single band in a GreenSafe Premium-stained agarose gel. Similar functional tests are performed with Speedy NZYTaq DNA polymerase buffer and $MgCl_2$ solution.

Troubleshooting

No product amplification or low yield
• Inadequate annealing temperature
The reaction mix composition may affect the melting properties of primers and DNA. Adjust the annealing temperature to accommodate the primer with the lowest melting temperature (5 °C to 10 °C lower than T_m).
• Presence of PCR inhibitors
Some DNA isolation procedures, particularly genomic DNA isolation, can result in the co-purification of PCR inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to the reaction. Diluting samples even 1:10,000 has been shown to be effective in improving results, depending on initial DNA concentration.
Presence of non-specific bands
• Non-specific annealing of primers
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.
• Mg^{2+} concentration is too high
Generally, 2-3 mM $MgCl_2$, typically 2.5 mM final concentration, works well for the majority of PCR reactions. Optimal concentration depends on target template, buffer and dNTPs. Optimize magnesium concentration by supplementing $MgCl_2$ in 0.5 increments up to 4 mM.

V2001

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Genomic DNA contamination	Pass
DNase contamination	Pass
Functional assay	Pass

Approved by:

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