NZYTaq II with 5× Gel Load Reaction Buffer

Catalogue number: MB36401, 500 U
MB36402, 1000 U
MB36403, 2500 U

Description

NZYTaq II DNA polymerase belongs to a new generation of *Taq*-derived DNA polymerases that was engineered to produce high DNA yields in shorter PCR running times (15-30 s/kb extension) under minimal optimization conditions. NZYTaq II DNA polymerase supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. NZYTaq II is provided with 5× Gel Load Reaction Buffer allowing reactions to be loaded directly into gels without the extra adding of loading dye. This Gel Load Reaction Buffer is composed by a blue and yellow dye. The blue dye migrates at the same rate as a 3-5 kb DNA fragment in a 1% (w/v) agarose gel. The yellow dye migrates at a rate faster than primers (<50 bp) in a 1% (v/v) agarose gel. The 5× Gel Load Reaction Buffer is not suitable when direct fluorescent or absorbance readings are required without prior purification of the amplified DNA from PCR. NZYTaq II DNA polymerase lacks 3´→5´ exonuclease activity. Resulting PCR products have an A overhang and are suitable for cloning with NZYTech´s TA PCR cloning kits (MB053 or MB137).

Storage temperature

NZYTaq II DNA polymerase should be stored at -20 °C in a constant temperature freezer. NZYTaq II 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

Gel Load Reaction Buffer (5×): Proprietary formulation supplied at pH 8.8. Vortex the reaction buffer thoroughly after thawing and prior to use. Repeated freeze-thaw cycles will affect the stability of the buffer (the buffer will remain stable at 4 °C for at least one month).

Magnesium Chloride solution: the provided 50 mM $MgCl_2$ solution allows users to optimize Mg^{2+} concentration in different PCR set ups. In general, NZYTaq II 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:

5× Gel Load Reaction Buffer	10 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)	5 pg-0.5 µg
NZYTaq II (5 U/µL)	0.25-1 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	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	94 °C	30 s	25-35
Annealing	*	30 s	
Extension	72 °C	15-30 s/kb	
Final Extension	72 °C	5-10 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.

4. Analyse PCR products through 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 15–30 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.

DNA template

The optimal amount of starting material may vary depending on the quality and complexity of template DNA. In general, we recommend using 20-500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify DNA fragments from as little as 5 pg of human gDNA, for example. Lower amounts of DNA template (typically 1-20 ng) may be used for amplification of lambda or plasmid DNA or even 5-20 ng for amplification of multicopy chromosomal genes. When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity
NZYTaq II DNA polymerase purity is >90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination
NZYTaq II 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 NZYTaq II DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with NZYTaq II buffer and $MgCl_2$ solution.

Functional assay
NZYTaq II DNA polymerase is extensively tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (1 and 2.5 kb) from human genomic DNA in the presence of 5× Gel Load Reaction Buffer and $MgCl_2$ solution. The resulting PCR products are visualized as single bands in a GreenSafe Premium-stained agarose gel.

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.
• Additives required
Adding PCR-enhancing agents (NZYTaq 5× Optimizer Solution – MB060 or NZYTaq 2× GC-Enhancer Solution – MB143) may improve yield while allowing the amplification of difficult templates.
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.

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Certificate of Analysis

Test	Result
Enzyme purity*	Pass
DNase contamination	Pass
Functional assay	Pass

*These assays were exclusively performed with the enzyme

Approved by: José Prates

José Prates
Senior Manager, Quality Systems

nzytech genes & enzymes
Estrada do Paço do Lumiar, Campus do Lumiar, Edifício E, R/C, 1649-038 Lisboa
Tel: +351.213643514 Fax: +351.217151168 www.nzytech.com