Hot-start NeoTaq II DNA polymerase

#Cat : NB-60-0008 Size : 500U

Description

Hot-start NeoTaq II DNA polymerase is an engineered version of NeoTaq II DNA polymerase displaying a hot-start-like PCR capacity. The enzyme activity at room temperature is limited, avoiding extension of non-specifically annealed primers or primer-dimers and thus providing higher specificity, sensitivity and yield during DNA amplification. The functional activity of the enzyme is restored during a short 5-minute incubation step at 95°C. Once Hot-start NeoTaq II DNA polymerase is activated, it displays high performance leading to high DNA yields in shorter extension times (15-30 s/kb) of DNA templates up to 6 kb. Hot-start NeoTaq II DNA polymerase requires minimal optimization. In addition, the hot- start-like capacity of Hot-start NeoTaq II DNA polymerase not only leads to higher PCR sensitivity but also allows a room-temperature reaction setup. Hot-start NeoTaq II DNA polymerase lacks 3'→5' exonuclease activity.
Resulting PCR products have an A-overhang and are suitable for cloning with PCR cloning kit.

Shipping conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage conditions

This product should be stored at -85 °C to -15 °C in a freezer without defrost cycles to guarantee maximal shelf life. The product 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 Mg^{2+} concentration in different PCR set ups, although the enzyme usually works effectively with a 2.5 mM $MgCl_2$ concentration. Prior to use, 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. In a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of PCR reactions. Set up the PCR reaction at room temperature.

A single reaction mixture of 50 µL should combine the following components: (**Note**: template DNA should be the last component to be added to the reaction mixture)

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)	1 pg-0.5 µg
Hot-start NeoTaq II DNA polymerase (5 U/µL)	0.25-1 µL
Nuclease-free water	up to 50 µL

(*) Final concentrations less than 0.25 µM may be beneficial to improve sensitivity in some conditions.

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	94 °C	30 sec **(¥)**	25-35
Annealing	**(*)**	30 sec	
Extension	72 °C	15-30 sec/kb **(¥)**	
Final Extension	72 °C	5-10 min	1

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

(¥) For DNA fragments higher than 3 kb to 6 kb in size, it may be beneficial to use 20 sec for denaturation and 30-60 sec/kb for extension.

4. Analyse the PCR products through agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with any Safe DNA Gel Stain.

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 (Tm), 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 its quality and complexity. In general, we recommend using 5 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 1 pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 0.5-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity

Hot-start NeoTaq II DNA polymerase purity is > 90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nuclease assays

0.2-0.3 µg of plasmid DNA are incubated with 5 U of Hot-start NeoTaq II DNA polymerase, in 1× Reaction Buffer, for 14- 16 hours at 37 °C. Following incubation, the DNA is visualised on a Safe DNA Gel stain-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with Hot-start NeoTaq II buffer and $MgCl_2$ solution.

Functional assay

Hot-start NeoTaq II DNA polymerase is extensively tested for performance in a polymerase chain reaction (PCR) of differentsized DNA fragments (1 and 2.5 kb) from human genomic DNA. The resulting PCR products are visualized as single bands in a any Safe DNA Gel stain-stained agarose gel. Similar functional tests are performed with Hot-start NeoTaq II 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 Tm).

- **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 (5× Optimizer Solution or 2× GC-Enhancer Solution) 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.

- **Mg2+ concentration is too high**

Generally, 1.5-3 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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