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BIOTOOLS

BIOTOOLS B&M LABS.S.A.

BIOTOOLS DNA POLYMERASE (1 U/μL)

| REF. | FORMAT | CONTENT |
|----------|-------------------|---|
| 10.002 | 500 U | Biotoools DNA Polymerase (1 U/μL) 10X Standard Reaction Buffer with MgCl ₂ |
| 10.003 | 1000 U | Biotoools DNA Polymerase (1 U/μL) 10X Standard Reaction Buffer with MgCl ₂ |
| 10.004 | 5000 U (5x1000 U) | Biotoools DNA Polymerase (1 U/μL) 10X Standard Reaction Buffer with MgCl ₂ |
| 10.012 | 500 U | Biotoools DNA Polymerase (1 U/μL) 10X Reaction Buffer MgCl ₂ FREE |
| 10.013 | 1000 U | Biotoools DNA Polymerase (1 U/μL) 10X Reaction Buffer MgCl ₂ FREE |
| 10.014 | 5000 U (5x1000 U) | Biotoools DNA Polymerase (1 U/μL) 10X Reaction Buffer MgCl ₂ FREE |
| 10.000BW | BULK | Biotoools DNA Polymerase (1 U/μL) |
| 10.000B | BULK | Biotoools DNA Polymerase (1 U/μL) 10X Standard Reaction Buffer with MgCl ₂ |
| 10.010B | BULK | Biotoools DNA Polymerase (1 U/μL) 10X Reaction Buffer MgCl ₂ FREE |

Store at -20°C

Notice to users: Some of the applications which may be performed with this product are covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application.

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1. GENERAL CONSIDERATIONS

BIOTOOLS DNA Polymerase is a modified thermostable recombinant DNA polymerase from the thermophilic bacterium *Thermus sp.* expressed in *E. coli*. The general characteristics of BIOTOOLS DNA Polymerase make the enzyme suitable for applications requiring a highly thermostable and processive enzyme capable of synthesising DNA strands at elevated temperatures in amplification or similar reactions (e.g. primer extension), thus resolving the most complex secondary structures.

Due to its processivity and accuracy BIOTOOLS DNA Polymerase allows the generation of long templates with a base misincorporation rate (1-10 x 10⁻⁶ bp) lower than most commercial *Taq* DNA polymerases.

The procedure employed for the purification of thermostable enzymes is proprietary of Biotoools. It involves a simple and non-chromatographic procedure which renders a top high yield and quality enzyme.

The enzyme is supplied at a concentration of 1 U/μL in a storage buffer. This concentration allows accurate pipetting of small amounts.

Product applications:

- Standard PCR
- Multiplex PCR
- In situ PCR
- DNA sequencing

2. ENZYME FEATURES

| | |
|--------------------------------------|-------------|
| Concentration:..... | 1 U/μL |
| Performance: | |
| Working concentration..... | 20-25 mU/μL |
| pH..... | 8-9 |
| Elongation temperature..... | 72°C |
| MgCl ₂ concentration..... | 2 mM |
| Size of PCR products:..... | Up to 5 kb |
| PCR cloning:..... | T/A |
| Endonuclease activity:..... | No |
| Reverse transcriptase activity:..... | No |
| 5'→3'exonuclease activity:..... | Yes |
| 3'→5'exonuclease activity:..... | No |
| Nicking activity..... | No |

This enzyme is not recommended for certain experiments dealing with amplification of sequences homologous to those found in *E. coli*.

3. STORAGE CONDITIONS

Store package components at -20°C in a constant temperature freezer. If stored under the recommended conditions, the product will maintain performance through the indicated date on the label.

4. PRODUCT SPECIFICATIONS

Unit Definition- One unit is defined as the amount of enzyme which incorporates 10 nano-moles of dNTPs into acid-insoluble DNA within 30 min at 72 °C.

Storage Buffer- 10 mM Tris-HCl (pH 8.0); 50 mM KCl; 1 mM EDTA; 0.1% Triton X-100; 50% glycerol (v/v).

10X Reaction Buffer- 750 mM Tris HCl (pH 9.0), 500 mM KCl, 200 mM (NH₄)₂SO₄. The 10X STANDARD REACTION BUFFER with MgCl₂ includes 20 mM MgCl₂ in its composition.

5. GENERAL ASPECTS OF REACTION COMPONENTS

Enzyme Concentration

As an initial guide we recommend employing the following enzyme units/rxn.

| Final reaction volume | Recommended enzyme units |
|-----------------------|--------------------------|
| 100 μL | Up to 2.5 U |
| 50 μL | 1-1.25 U |
| 25 μL | 0.5-0.625 U |

The addition of higher quantities of enzyme generally does not produce significant yield increase. Only for certain applications or when working on long DNA fragment amplifications (longer than 2 kb from genomic DNA) it might be necessary to increase the concentration.

DNA Template

The quality and quantity of the DNA template affects both the sensitivity and efficiency of the amplification. High amounts of DNA usually increase the amplification of nonspecific PCR products.

The PCR is inhibited by various compounds e.g. ionic detergents, phenol, gel loading dyes, etc. If the template contains traces of inhibitors, reduce the amount of the DNA included in the amplification reaction, or repurify the template by ethanol precipitation.

dNTPs Concentration

Generally equal concentrations of all four dNTPs are used. The concentration of each dNTP should be 50-500 μM, being 200 μM the most commonly used concentration. Biotoools offers equimolar mixes of dNTPs (10 mM and 25 mM each).

The concentration of dNTPs may be decreased (e.g. when unspecific amplification occurs), increased (e.g. for long amplifications), or even unbalanced in favour of any of the particular dNTPs (e.g. in vitro mutagenesis experiments). Biotoools DNA Polymerase is suitable for its use with modified dNTPs (e.g. radioactively or fluorescein labelled) as substrates. It can also be used with dUTP and other analogues.

The dNTPs behave as potent Mg²⁺ chelating agents reducing therefore the availability of free Mg²⁺ for polymerase activity. Thus an increase in dNTPs should be accompanied by an increase in MgCl₂ concentration.

Reaction Buffer

The provided buffer has been specially formulated to facilitate the amplification of any PCR products. It creates the appropriate stringent conditions for primer-annealing over a wide range of temperatures. Moreover, the Standard Reaction Buffer with MgCl₂ includes Mg²⁺ at the optimal concentration for most experiments (final concentration: 2 mM).

MgCl₂ Concentration

The optimal MgCl₂ concentration may vary depending on the primer and template that are used and must be determined by experimentation. In most cases, a final concentration of MgCl₂ at 2 mM in the reaction mix works well.

High concentrations of MgCl₂ may promote low enzyme fidelity and non specific amplification products; whereas low concentrations should reduce the yield of the desired amplification products. If the samples contain any chelating metal agents such as EDTA, the concentration of MgCl₂ should be increased accordingly.

Primer Design

PCR primers are usually 15-30 nucleotides in length, with a content of 40-60% G+C residues. To avoid primer-dimer and hairpin formation the primers should not be self-complementary or complementary to any other primer present in the reaction mixture. The annealing temperature of the primers should be similar (< 5°C variation). Length and G+C content of primers are used to predict their annealing temperature to the template DNA. The 5' end of a primer may contain mismatches between the primer and template, whereas this is not recommended at the 3' end.

PCR Additives

In certain cases the presence of DMSO, betaine, formamide or any other PCR additives might be necessary for optimized complex PCR reactions. The provided enzyme and buffer are compatible with most PCR additives. When calculating the annealing temperature for the PCR cycling program, it is important to take into account that certain additives may decrease the melting temperature of the primers.

6. STANDARD PROTOCOL

Optimal conditions must be determined for each individual experimental system.

Proceed to the Reagent Preparation Area in a laminar flow cabinet. Wear disposable gloves and use sterile and nuclease free plastic material in order to avoid contaminations and false negative results.

1. Thaw reagents on ice. After complete thawing, mix the reagents well, spin down in a bench-top centrifuge and keep on ice.

2. Prepare a master mix in a sterile microcentrifuge tube according to Table 1. For each experiment include at least one negative control (without template DNA). To ensure sufficient volume for all desired reactions include additional reactions in the calculations.

TABLE 1. Master Mix preparation

| COMPONENT | Final Concentration | 50 µL rxn | 20 µL rxn |
|-----------------------------------|---------------------|-----------------|-----------------|
| Master Mix | | | |
| 10X REACTION BUFFER | 1X | 5 µL | 2 µL |
| 50 mM MgCl ₂ solution* | 1.5-4 mM | 1.5-4 µL | 0.6-1.6 µL |
| dNTP Mix 10 mM each | 200 µM of each | 1 µL | 0.4 µL |
| Primers | variable | variable | variable |
| DNA Polymerase (1 U/µL) | 20-25 mU/µL | 1-1.25 µL | 0.4-0.5 µL |
| Sterile bidistilled water | - | Up to 50 µL | Up to 20 µL |
| Template DNA | Variable | Variable | Variable |

*not necessary for 10X Standard Reaction Buffer because it includes MgCl₂

3. Mix the master mix thoroughly and keep on ice. Distribute the appropriate volume into each vial.

Proceed to DNA Purification Area separate from other sources of DNA.

4. Add the template DNA to each reaction vial. Close the vials and mix gently. For thermal cycler without heated lid overlay a mineral oil layer.

Proceed to the Amplification Area

5. Program the thermal cycler according to the guide of the amplification program (see Table 2 and Section 7). Place the vials in the thermal cycler and perform the selected PCR program.

TABLE 2. Standard Amplification Program*

| CYCLE STEP | Nº CYCLES | TEMPERATURE | TIME |
|----------------------|-----------|---------------------|-------------|
| Initial Denaturation | 1 | 94°C | 3-10 min** |
| Denaturation | 25-35* | 94°C | 5-60 sec |
| Annealing | | T _m -5°C | 30-60 sec |
| Extension | | 72°C | 60 sec/1 kb |
| Final Extension | 1 | 72°C | 5-15 min |
| Cooling | ∞ | 4°C | ∞ |

*Optimize the time, the temperature and the number of cycles of the PCR.

**Depending on the template

7. GUIDE TO AMPLIFICATION PROGRAM

Initial Denaturation Step-Incomplete denaturation of the PCR reaction results in an inefficient first amplification cycle and low amplification yield. However, the denaturation must be kept as short as possible in order to avoid inactivation of the enzyme. For most samples 94°C for 3-5 min should be satisfactory; templates rich in G+C often require a longer step (up to 10 minutes).

Denaturation Step-The PCR product synthesized in the amplification cycling is shorter than the template DNA and therefore needs a short denaturation step; 5-60 sec of denaturation at 94°C should be sufficient.

Primer Annealing Step-In general for primers < 20 bases the optimal annealing temperature is equal to the T_m of the lowest T_m primer. To find the optimal annealing temperature, you can use a temperature gradient. Start using an annealing temperature 5 °C below the T_m of the primers. If primers have a high T_m a two-step cycling is recommended.

Extension Step-The annealed primers must be extended at 70-75°C. The extension time depends on the size of the expected product. For Biotools DNA Polymerase we recommend 1 min for each kb of expected product.

Number of PCR Cycles-Cycling program usually consists on 25-35 cycles. This parameter depends on the amount of starting material and the expected yield. In certain experiments, increasing the number leads to an increase in nonspecific products. You should experimentally determine the optimal number of cycles.

Final Extension Step-After the last PCR cycle the sample should be incubated at 72°C for 5-15 min. The DNA polymerase fills the protruding ends of the newly synthesized PCR products and adds extra adenine nucleotides to the 3' ends of the PCR product.

8. TROUBLESHOOTING

| Problem | Cause | Recommendation |
|--|------------------------------------|---|
| Low yield or no amplification product | Missing reagent or pipetting error | Check concentration and storage conditions of dNTPs, primers, etc. Repeat the PCR. |
| | DNA template problems | Check the concentration and quality of starting material. If the template is difficult e.g. rich in G+C sequences we recommend adding DMSO to the master mix. Repeat the PCR with a new dilution of template or with a new DNA purification. |
| | Problems with primers | Revise the primers design and the primers storage condition. Avoid any design prone to the formation of primer dimers. Repeat PCR with different primer concentration from 0.1-0.5 µM in 0.1 increments. Check primer degradation on a denaturing polyacrylamide gel. |
| | Enzyme concentration too low | Increase enzyme concentration in 0.2 U increments. |
| | MgCl ₂ concentration | Optimise MgCl ₂ concentration of the PCR if necessary (1.5-4 mM) |
| | Incorrect PCR cycling conditions | Check the following parameters of the PCR program: Denaturation - Increase time and temperature of initial denaturation. Annealing - Decrease the annealing temperature and optimise time. Extension time - Increase extension time by increments of 30 sec. Number of cycles -Perform additional cycles by increments of 5 cycles. Verify the final elongation step. |
| Nonspecific amplification products or background smear | Annealing temperature too low | Increase the annealing temperature in increments of 1°C. |
| | Problems with primers | Design alternative primers. Both primers should be present at the same concentration (0.1-0.5 µM). Decrease primer concentration by decrements of 0.1 µM. Check primer degradation on a denaturing polyacrylamide gel. |
| | Excess of DNA template | Use dilutions of your template. |
| | Enzyme concentration too high | Optimise polymerase concentration of the PCR if necessary |
| | MgCl ₂ concentration | Optimise MgCl ₂ concentration of the PCR if necessary (1.5-4 mM) |
| PCR products in negative control | Incorrect PCR cycling conditions | To increase the specificity you can perform a touchdown or step-down PCR. Reduce the number of cycles. Increase the annealing temperature in increments of 1°C. |
| | Carryover contamination | Exchange all reagents. |

9. ORDERING INFORMATION

| Components | References | | | | | | | | |
|---|------------|-----------|------------|-----------|-----------|------------|------------|------------|------------|
| | 10.002 | 10.003 | 10.004 | 10.012 | 10.013 | 10.014 | 10.000BW | 10.000B | 10.010B |
| Biotools DNA Polymerase (1 U/µL) | 500 U | 1000 U | 5 x 1000U | 500 U | 1.000 U | 5 x 1000U | On request | On request | On request |
| 10X Standard Reaction Buffer with MgCl₂ | 2 x 1.8ml | 3 x 1.8ml | 15 x 1.8ml | | | | | On request | |
| 10X Reaction Buffer MgCl₂ FREE | | | | 2 x 1.8ml | 3 x 1.8ml | 15 x 1.8ml | | | On request |
| 50 mM MgCl₂ Solution | | | | 1 x 1.8ml | 2 x 1.8ml | 10 x 1.8ml | | | |