Produced by:
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BIOTOOLS
BIOTOOLS B&M LABS.S.A.

BIOTOOLS ULTRATOOLS DNA POLYMERASE (1U/µl)

REF.	FORMAT	CONTENT
10.221	100 U	**Biotools Ultratools DNA Polymerase (1 U/µl)** 10X Standard Reaction Buffer with $MgCl_2$
10.222	250 U	**Biotools Ultratools DNA Polymerase (1 U/µl)** 10X Standard Reaction Buffer with $MgCl_2$
10.231	100 U	**Biotools Ultratools DNA Polymerase (1 U/µl)** 10X Reaction Buffer $MgCl_2$ FREE
10.232	250 U	**Biotools Ultratools DNA Polymerase (1 U/µl)** 10X Reaction Buffer $MgCl_2$ FREE

Store at -20ºC

Research Use Only. Not for use in diagnosis procedures

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. DESCRIPTION

Biotools Ultratools DNA Polymerase is a modified thermostable recombinant DNA polymerase from the thermophilic bacterium *Thermus sp.* This polymerase is suitable for applications which require a highly pure polymerase and it is the enzyme of choice for applications involving bacterial DNA sequences homologous to *E. coli.*

Since this enzyme is virtually free of bacterial DNA, pathogen DNAs in low copy number are effectively amplified from biological samples. Therefore, this polymerase enhances PCR product yield without altering the fidelity of DNA replication and can also be used to amplify complex genomic targets.

As a result, the use of *Biotools Ultratools DNA Polymerase* facilitates detection of genetic diseases or infections, allowing an easier PCR-based screening for identifying large-scale diagnostic bacteria as well as other types of screening requiring high throughput PCR.

Due to its processivity and accuracy *Biotools Ultratools 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.

Product applications:

- Standard PCRs
- Multiplex PCRs
- In situ PCRs
- DNA sequencing

2. ENZYME FEATURES

Working concentration	20-25 mU/µl
Extension rate:	1 kb/min at 72ºC
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

3. STORAGE CONDITIONS

Store the product at **-20ºC** in a constant temperature freezer until the expiration date printed on the label.

Avoid exposure to frequent temperature changes.

4. PRODUCT SPECIFICATIONS

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

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 $(NH_4)_2SO_4$. The **10X STANDARD REACTION BUFFER with $MgCl_2$ includes 20 mM $MgCl_2$** in its composition**.**

5. GENERAL CONSIDERATIONS

Enzyme Concentration

Biotools Ultratools DNA Polymerase is suitable for standard and specialized PCR applications. We recommend adding 20-25 mU/µl of enzyme; only for specific applications or when working on long DNA fragment amplifications (longer than 2 kb from genomic DNA) might it 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. The PCR is inhibited by various compounds e.g. ionic detergents, phenol, gel loading dyes, etc. If the DNA template contains traces of inhibitors, reduce the amount of the template included in the amplification reaction, or re-purify the template by ethanol precipitation and several washing steps.

The *primer: template ratio* strongly influences the specificity and efficiency of PCR and should be optimised empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events.

dNTPs Concentration

Generally equal concentrations of all four dNTPs are used. The concentration of each dNTP should be 50-500 µM, 200 µM being the most commonly used concentration. The concentration of dNTPs may be decreased (e.g. when non-specific amplification occurs), or increased (e.g. for long amplifications).
The dNTPs behave as potent Mg^{2+} chelating agents reducing therefore the availability of free Mg^{2+} for polymerase activity. Thus an increase in dNTPs should be accompanied by an increase in $MgCl_2$ concentration.

$MgCl_2$ Concentration

The optimal $MgCl_2$ concentration may vary depending on the primer and template used and must be determined by experimentation. The *Standard Reaction Buffer with $MgCl_2$* includes Mg^{2+} at the optimal concentration for most experiments (final concentration: 2 mM) which facilitates the use of the enzyme.

Reaction Buffer

The buffer provided has been specially formulated to facilitate the amplification of any PCR product. It creates the appropriate stringent conditions for primer-annealing over a wide range of temperatures.

Primer Design

PCR primers are usually 15-30 nucleotides in length with 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 (< 5ºC variation). Length and G+C content must be selected accordingly.

PCR Additives

In certain cases the presence of DMSO, betaine, formamide or any other PCR additives might be necessary for optimised 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.

1. Thaw reagents on ice. After complete thawing, mix the reagents well, spin down 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).

Table 1. Master Mix preparation

COMPONENT	Final Concentration	50 µl rxn
Master Mix		
Biotools Ultratools DNA Polymerase (1U/µl)	20-25 mU/µl	1-1.25 µl
10X Reaction Buffer*	1 X	5 µl
50mM $MgCl_2$ Solution	1.5-4mM	1.5-4 µl
dNTP Mix 10 mM each	200 µM each	1 µl
Primer A	0.1-0.5 µM	x µl
Primer B	0.1-0.5 µM	x µl
Template DNA	variable	variable
Sterile bidistilled water	-	Up to 50 µl

*10X Standard Reaction Buffer includes $MgCl_2$

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 (< 500 ng/reaction) to each reaction vial. Close the vials and mix gently.

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	94ºC	3-5 min*
Denaturation Annealing Extension	25-35	94ºC T_m-5ºC 72ºC	5-60 sec 30-60 sec 60 sec/1 Kb
Final Extension	1	72ºC	5-15 min
Cooling	∞	4ºC	∞

**Depending on the template*

7. GUIDE TO AMPLIFICATION PROGRAM

Initial Denaturation Step-Incomplete denaturation step 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 94ºC for 3-5 minutes should be satisfactory; templates rich in G+C content often require longer initial denaturation and the length of this step can be extended (≤ 10 min).

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 94ºC should be sufficient.

Primer Annealing Step-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 a temperature 5 ºC < T_m of the primers. If the primers have a high T_m, two step cycling is recommended.

Extension Step-The annealed primers must be extended at 70-75ºC. The extension time depends on the size of the expected product. Recommended extending time for *Biotools Ultratools DNA Polymerase* is 1 min/kb of amplicon.

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

Final Extension Step-After the last PCR cycle the sample should be incubated at 72ºC 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 products.

8. TROUBLESHOOTING

Little or no amplification detected

1. **Problems with template.** Check the quality and quantity of template DNA by agarose gel electrophoresis or fluorimetry. Organic extraction followed by ethanol precipitation may remove some inhibitors. Use of excess template can reduce PCR product yield.

   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.
2. **Problems with primers.** *Design primers* that have higher annealing temperatures and do not form hairpin loops or primer-dimers.

   Check *primer degradation* on a polyacrylamide gel.

   Although lower *primer concentration* can prevent primer-dimer formation, sufficient primers are needed for successful PCR. Increase primer concentration in increments of 0.1 µM.
3. **Enzyme concentration not optimal.** Increase the enzyme in 0.2 U increments.
4. **$MgCl_2$ concentration too low.** Optimise Mg^{2+} concentration between 1.5-4 mM.
5. **PCR cycling conditions not optimal.**
   Increase the length of *initial template denaturation* up to 8 minutes.

   Lower the annealing temperature in 2°C decrements.

   Perform *additional cycles* in increments of 5 cycles.

   Increase the extension time by increments of 30 sec. Generally 60 seconds/kb of PCR product should be enough.
6. **Pipetting error or missing reagent.** Repeat PCR. Check the concentration and storage conditions of reagents.

Multiple products or a smear detected

1. **Too much template.** Check the concentration of template DNA by agarose gel electrophoresis or fluorimetry. Decrease the amount of DNA added to the reaction.
2. **Enzyme concentration not optimal.** Decrease the enzyme in 0.2 U decrements.
3. **$MgCl_2$ concentration too high.** Decrease Mg^{2+} concentration in the reaction.
4. **Problems with primers.** Check *primer degradation* on a polyacrylamide gel.

   Use specific software for *primer design*.

   Decrease the *amount of primer* added to the reaction.
5. **PCR cycling conditions not optimal.**
   Increase the annealing temperature in 2°C increments.

   Decrease number of cycles in decrements of 5 cycles.
6. **Carryover contamination.** If negative control (without DNA) shows a PCR product or smear, exchange all reagents.

9. ORDERING INFORMATION

DESCRIPTION	Size	Reference
Biotools Ultratools DNA Polymerase (1U/µl)	110 µl	10.221
	260 µl	10.222
	110 µl	10.231
	260 µl	10.232
10X Standard Reaction Buffer with $MgCl_2$	1.8 ml	10.221
	1.8 ml	10.222
10X Reaction Buffer $MgCl_2$ FREE	1.8 ml	10.231
	1.8 ml	10.232
50mM $MgCl_2$ Solution	1.8 ml	10.231
	1.8 ml	10.232