Produced by:
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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
10.220BW	BULK	**BIOTOOLS Ultratools DNA Polymerase (1 U/µl)**
10.220B	BULK	**BIOTOOLS Ultratools DNA Polymerase (1 U/µl)** 10X Standard Reaction Buffer with $MgCl_2$
10.230B	BULK	**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.

Ed .10 – June 2023

1. DESCRIPTION

Biotools Ultratools DNA Polymerase is a modified thermostable recombinant DNA polymerase from the *Thermus sp* further purified through a manufacturing process to reduce bacterial DNA introduced from the host. The purification process ensures that non-specific or false positive products, due to DNA contamination, are minimized during the PCR. This enzyme is a highly purified version of Biotools DNA Polymerase. The enzyme is tested for protein purity and the absence of DNA (see Purity Test).

The Ultratools DNA Polymerase from Biotools facilitates detection of infections and genetic diseases in biological samples.

*Ultratools DNA Polymerase is supplied at a **concentration of 1 U/µl** in a storage buffer. This concentration allows accurate pipetting of small amounts of the DNA polymerase, so that further dilutions are not necessary.*

Product applications:

- *Highly specific amplification*
- *Low-copy target amplification*
- *Bacterial sequences amplification*
- *Detection of bacterial pathogens in clinical samples*
- *Standard PCR*
- *qPCR*

***Purity test*:** Lot quality assurance sampling uses primers targeting highly conserved region in the 16S ribosomal gene, in the absence and presence of template DNA.

2. ENZYME FEATURES

Working concentration	10-40 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, and 50% glycerol (v/v).

10X Reaction Buffer- 750 mM Tris-HCl (pH 9.0), 500 mM KCl, and 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 20-40 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. 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 DNA template contains traces of inhibitors, reduce the amount of the template included in the amplification reaction, or repurify the template by ethanol precipitation and several washing steps.

dNTPs Concentration

The concentration of each dNTP in amplification reactions should be 50-500 µM, being 200 µM the most commonly used concentration. 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).

The dNTPs behave as potent Mg^{2+} chelating agents; 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. The **Standard Reaction Buffer includes Mg^{2+}** at the optimal concentration for most experiments (final concentration: 2 mM) which facilitates the use of the enzyme.

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 complex amplifications the presence of DMSO, betaine, formamide or any other PCR additives might be necessary. 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

Note 1: *Due to the exponential amplification that occurs during PCR, scarce amount of contaminant DNA can result in product formation even in the absence of template. Using* ***Biotools Ultratools DNA Polymerase****, is only one key to attaining very low background level of bacterial DNA. To exploit the high purity of this product, you must take care to ensure that all PCR reagents and supplies are free of background DNA and that PCR amplifications occur in a DNA-free environment.*

Take care to separate areas of DNA preparation from areas where PCR reagents are handled for runs, in particular preparation of master mixes, pipetting into tubes and performance of PCR runs. Take care to avoid cross contamination by aerosols. Use sterile and nuclease free plastic material in order to avoid contaminations. Wear disposable gloves and lab coats.

Optimal conditions must be determined for each individual experimental system.

Proceed to the Reagent Preparation Area in a laminar flow cabinet.

1. Thaw and keep reagents on ice. After complete thawing, mix the reagents and spin down.

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)	10-40 mU/µl	0.5-2.0 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 nuclease-free 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 Point 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 (see Point 7).*

*** Optimize time; temperature; and number of cycles (see Point 7).*

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- To find the optimal annealing temperature, you can use a temperature gradient. Start using an annealing temperature 5 ºC below the T_m of the primers. If the primers have a high T_m a two-step cycling is recommended.

Extension Step-The annealed primers must be extended at 70-74ºC. 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 Ultratools 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.
   Repeat the PCR with a new dilution of template or with a new DNA purification.
   If the template is difficult e.g. rich in G+C sequences, we recommend adding DMSO to the master mix.
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, repeat the assay following the instructions of Note 1.

9. ORDERING INFORMATION

Components	**References**						
	10.221	**10.222**	**10.231**	**10.232**	**10.220BW**	**10.220B**	**10.230B**
Biotools Ultratools DNA Polymerase (1 U/µl)	100 U	250 U	100 U	250 U	On request	On request	On request
10X Standard Reaction Buffer with $MgCl_2$	1.8ml	1.8ml				On request	
10X Reaction Buffer $MgCl_2$ FREE			1.8ml	1.8ml			On request
50 mM $MgCl_2$ Solution			1.8ml	1.8ml			