

Taq DNA Polymerase, 500U

Code	Description	Size
N224-500U	Taq DNA Polymerase, 500U	200 reactions

General Information

The PCR (Polymerase Chain Reaction) is a widely used molecular biology application for the rapid amplification (duplication) of specific gene sequences. The DNA to be amplified is added to a solution containing the following components: primers, dNTPs, DNA Polymerase, and reaction buffer containing a divalent cation (Mg^{2+} , Mn^{2+}). A typical PCR reaction consists of three steps that combine to form one doubling cycle. In the first step, denaturation, the reaction is heated to 95°C to relax the DNA template and separate the double-stranded helix. The reaction is then cooled to 35 – 72°C for the annealing (binding) step, which allows the primers to bind to the recognition sequence of the template. In the final, elongation step, Taq polymerase extends from the 3' end of the primer at 72°C to form a newly synthesized “daughter” strand. The cycle then repeats itself for a total of 25 – 50 times and results in an exponential increase in the concentration of double-stranded DNA template.

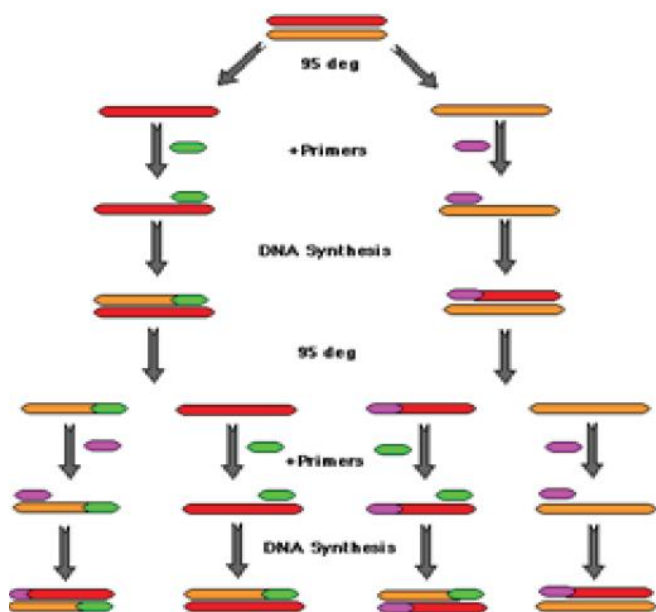


Figure 1. Exponential increase in double stranded DNA molecules by the Polymerase Chain Reaction

Storage/Stability

Taq DNA Polymerase is stable for 1 year when stored frozen (-15 to -22°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Materials Supplied

Taq DNA Polymerase, 500U

Taq Buffer, 10X: 1 mL of 160 mM (NH₄)₂SO₄, 500 mM Tris-HCl, pH 9.2 at 22°C, 17.5 mM MgCl₂, 0.1% Triton X-100

Required Materials Not Supplied

- Thin walled PCR tubes or a 96-well plate
- Template DNA
- Primers
- Thermocycler
- Ice
- Vortex
- Centrifuge

Protocol/Procedure:

Note: Reaction solutions should be assembled on ice.

1. Label thin-walled PCR tubes or a 96-well plate as needed.
2. To perform multiple parallel reactions, prepare a master mix consisting of dNTPs, buffer (MgCl₂, if necessary) and polymerase. Consult Table 1 to determine the volume of each component for a 25 µL reaction. Prepare a 10 – 20% overage to account for pipetting losses.

Reaction Component	Volume
Template DNA	1 – 3 µL (50 – 150 µg)
Taq Buffer, 10X	2.5 µL
dNTPs, 10 mM	0.5 µL
Forward primer, 20 µM	0.6 µL
Reverse primer, 20 µM	0.6 µL
Nuclease-free water	As needed to bring final volume to 25 µL
Taq DNA Polymerase, 500U	0.5 µL

Table 1

3. Aliquot master mix to tubes or wells and add DNA templates and primers. See Table 1 for recommended volumes of each.
4. Gently vortex samples and briefly centrifuge to collect reaction mix in bottom of tube.
5. Place in thermocycler and begin PCR. See Table 2 on the next page for cycling specifications. Cycling protocol may need to be optimized depending on sample/primer properties.

Step	Temperature (°C)	Time	Number of Cycles
Initial Denaturation	95	5 minutes	1
Denaturation	95	15 seconds	30
Annealing	50	30 seconds	
Extension	69	3 minutes	
Final Extension	69	7 minutes	1

Table 2

Table 2 Notes: During the final elongation step, the terminal transferase activity of Taq DNA Polymerase will incorporate an additional adenosine nucleotide to the 3' ends of the resulting amplicons. If the PCR fragments are to be TA cloned, this extension step can be extended for up to 30 minutes.

Frequently Asked Questions

Why do I have low or no yield?

1. Insufficient number of cycles. Place PCR vials back in thermocycler and run an extra 5 cycles.
2. DNA template denatured. Check DNA quality on agarose gel.
3. Thermocycler program not correct. Check temperature and cycle times.
4. Inhibitors present in reaction. Precipitate the original samples or purify over columns. Ions, such as EDTA may inhibit PCR reaction.
5. Missing reaction component. Check components and set up new reactions.
6. Unsuitable reaction conditions. Decrease annealing temperature and/or increase elongation time.
7. Samples might have evaporated. Cover reactions with high quality mineral oil or use a thermocycler with a heated lid.
8. Bad primers, ensure that the 5' and 3' ends of primers are not complementary. A high GC content or extremely mismatched Tm's may also have an effect on binding efficiency.
9. Incorrect primer specificity. Check sequences.
10. Primer concentrations are too low. Increase if necessary.
11. Bad dNTPs. dNTP solutions should be stored frozen with limited freeze/thaw cycles. Replace nucleotide solution if necessary.
12. Target not present in DNA template. Try another region in DNA template.

Frequently Asked Questions Continued

Why did I obtain a non-specific product(s)?

1. Sub-optimal reaction conditions. Optimize MgCl_2 concentration, annealing temperature, elongation time, and number of cycles. Keep reactions on ice when all reagents are mixed.
2. Poor primer design. Re-design primers. Ensure ends are not complementary and do not contain three successive G's or C's at the 3' end.
3. Primer concentration too high, lower if necessary.
4. Contamination with other template. Use dedicated pipettes and tips. Work in separate rooms or clean areas.
5. Several targets present with same target sequence. Develop new primers and run BLAST search in public database to ensure primer specificity.

For Technical Support

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