



**Catalog No. 11630-004**    **400 units**  
**Catalog No. 11630-020**    **2,000 units**  
**Catalog No. 11630-040**    **4,000 units**

## Taq DNA Polymerase

Store at -20°C

### Product Description

*Taq* DNA Polymerase is a thermally stable, processive, 5'-3' DNA polymerase. The 94 kDa protein possesses an inherent 5'-3' nick-translation moiety and lacks a 3'-5' proofreading function.

### Applications

Marligen's *Taq* DNA polymerase has been optimized for use with Marligen's Signet™ Genotyping Panels formatted on the Luminex platform. Other applications include PCR, Primer Extension, Microarray Analysis, High-throughput PCR, and Colony PCR.

### Source of Protein

A recombinant *E. coli* strain carrying the *Taq* DNA polymerase gene from the thermophilic organism *Thermus Aquaticus* YT-1.

### Components included with this product

Component Name	11630-004	11630-020	11630-040
Taq DNA polymerase supplied in 20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween-20, 0.5% Nonidet P-40, 50% Glycerol.	80 µL (5 U/µL)	400 µL (5 U/µL)	800 µL (5 U/µL)
10X PCR Buffer 1 containing 100 mM Tris-HCl, pH 8.6, 15 mM MgCl <sub>2</sub> , 750mM KCl.	2 x 1 mL	10 mL	2 x 10 mL

### Storage Conditions

Store at -20°C.

### Concentration

5 units/µL

### Unit Characterization Assay

Specific activity is measured using a 2-fold serial dilution method. Dilutions of enzyme batch are made in a reduced-glycerol (5%) containing *Taq* DNA Polymerases storage solution ([*Taq*]<sub>f</sub> = 0.009-0.0001µg/µL) and added to 50 µL reactions containing 12.5 µg Calf Thymus DNA, 25 mM TAPS (pH 9.3), 50 mM KCl, 1 mM DTT, 4mCi/mL <sup>3</sup>H-dTTP and 200 µM dNTPs. Reactions are incubated 10 minutes at 74°C, plunged on ice, and analyzed using the method of Sambrook and Russell.

### Quality Assurance

Purified free of contaminating endonucleases and exonucleases. In addition, enzyme purity is analyzed by SDS-PAGE.

### Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

### PCR Guidelines

*Taq* DNA Polymerase is the original and most commonly used PCR enzyme. *Taq* excels at amplifying shorter (<5 kb) sequences from low-complexity template sources and produces robust yields with little or no optimization of reaction conditions. Consider the following guidelines when designing PCR strategies using *Taq* DNA Polymerase.

- DNA Template:** Although extensive purification of PCR templates is typically not necessary, care should be taken with crude or partially purified DNA sources as handling and chemical agents can adversely affect the PCR process. Exposure to short-wave UV light or other DNA damaging agents should be avoided, as should high ionic strength, detergents such as SDS, loading dyes and phenol. In order to prevent contamination from previous PCR reactions, consider setting up reactions in a positive-pressure hood and with aerosol barrier pipet tips. In a typical 25 cycle PCR, 10<sup>4</sup> copies of target sequence will yield reproducible amplification product. This corresponds to roughly 0.1-1 ng/mL (final concentration) of plasmid DNA, and 1-10 µg/mL of genomic DNA. The use of lower DNA concentrations typically produces less non-specific product, while higher concentrations can allow for fewer cycles and lower mutation rates.
- Primer Design:** Ideally, oligonucleotide primers are 15-30 bases in length, nearly 50% G+C, and have equal (+/- 3°C) annealing temperatures. The use of software to detect self-complementary or hairpin-prone regions is advised and is offered as a service by some synthesis providers. Note that although the 5'-terminus of the primer may contain untemplated sequence, the 3' end must match perfectly. Typical oligonucleotide concentration in the reaction is 0.1-0.5 µM.
- Magnesium:** Magnesium is a critical component of the PCR reaction though its concentration can be modulated to promote various effects. Generally, 1.5-2.0 mM Mg<sup>2+</sup> is targeted, but higher concentrations (up to 5 mM) may be used to stimulate the yield of reactions at the expense of fidelity. The converse is also true – lower magnesium concentrations will promote higher- fidelity products with a lower overall amplification yield. Note that certain reaction components, in particular template DNA and oligonucleotides, may contribute chelating agents to the reaction which could lower the effective magnesium concentration and starve the reaction.

4. **dNTPs:** Generally, a final concentration of 100-200  $\mu\text{M}$  dNTPs is employed, though higher concentrations may stimulate yields (particularly with longer targets) and lower may offer increases in fidelity. *Taq* DNA Polymerase can also incorporate and read through deoxy Uridine and Inosine, two analogs used in certain applications.

5. ***Taq* Polymerase:** 1 unit/50  $\mu\text{L}$  reaction (20 U/mL) is typical, though additional enzyme may be added to stimulate yields. *Taq* DNA Polymerase extends a DNA template at approximately 2000 nucleotides/minute, so it is recommended that 45-60 seconds of extension time be provided per cycle. Appropriate extension temperatures range from 66-72°C.

6. **Reaction Set-Up:** When using *Taq* DNA Polymerase, reactions should be assembled on ice and *Taq* Polymerase added to the system last. A good practice, particularly when handling large numbers of similar samples, is to create two 2X master mixes – one containing *Taq* DNA Polymerase and buffer, and the other containing dNTPs, DNA, and primers. The DNA/primer mix should be mixed thoroughly and added to reaction vessels, and the polymerase/buffer mix similarly mixed and added to the DNA cocktail. Reactions can then be added to an appropriate thermal cycler for completion of the reaction. An alternate strategy is to assemble all components except the polymerase on ice, then place the tubes in the thermal cycler and raise the temperature to 94°C, then add the *Taq* enzyme and mix thoroughly. This approach provides the most assurance that no undesired reaction will occur prior to the initial denaturation step.

### Typical 50 $\mu\text{L}$ Reaction

On ice, prepare each of following master mixes, combine, and place in heated (to 94°C) thermal cycler

#### 2X DNA/Oligonucleotide Master Mix:

- 1.0  $\mu\text{L}$  10 mM dNTPs
- 1.0  $\mu\text{L}$  10  $\mu\text{M}$  Forward Primer
- 1.0  $\mu\text{L}$  10  $\mu\text{M}$  Reverse Primer
- 1.0  $\mu\text{L}$  500 ng/ $\mu\text{L}$  genomic DNA
- 21  $\mu\text{L}$  Nuclease free Water

#### 2X Enzyme/Buffer Master Mix:

- 5.0  $\mu\text{L}$  10X PCR Buffer I
- 0.2  $\mu\text{L}$  5 U/ $\mu\text{L}$  *Taq* DNA Polymerase
- 19.8  $\mu\text{L}$  Nuclease free Water

### General Cycling Conditions

Temperature	Time	Step
94°C	3 minutes	Initial Denaturation
25 Cycles:		
94°C	30 seconds	Denaturation
55°C	30 seconds	Annealing
68°C	30 seconds	500 bp extension
68°C	5 minutes	Final Extension

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<b>25 Cycles:</b>		
94°C	30 seconds	Denaturation
55°C	30 seconds	Annealing
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68°C	5 minutes	Final Extension

### References

Sambrook and Russell Molecular Cloning, vol3, 2001, pp. A8.25-A8.26.

### Legal Disclaimers

Certain applications in which this product can be used may be covered by patents issued and applicable in the United States and abroad. Purchase of this product does not include a license to perform any patented application, therefore it is the sole responsibility of users of this product to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used.