# Taq DNA Polymerase (5 U/µL)

## Product Description

Taq DNA Polymerase is a thermostable DNA Polymerase that catalyzes 5'→3' DNA synthesis. Taq DNA Polymerase has 5'→3' exonuclease activity and is deficient in 3'→5' exonuclease activity. Taq DNA polymerase amplifies uracil-containing templates, can incorporate modified bases, and performs A-tailing on DNA products. This enzyme is provided in a non-hot start format.

## Product Applications*

- PCR amplification of DNA fragments (≤5 kb)
- Probe and intercalating dye-based qPCR
- RT-qPCR
- Pre-ligation (dA tailing)
- Homebrew library prep

*Watchmaker Genomics has not tested or validated Taq DNA Polymerase in all applications listed.

## Unit Definition and Buffer Composition

- One unit of Taq DNA polymerase is the amount of enzyme that will incorporate 10 nmol of dNTP into activated calf thymus DNA in 30 minutes at 75°C
- Enzyme Storage Buffer: 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.5% Tween 20
- Standard 10X reaction buffer (not supplied with Kit): 200 mM Tris-HCl, pH 8.3, 400 mM KCl

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## Storage and Handling

Taq DNA Polymerase Kits are shipped on ice packs. Upon receipt, store all kit components at -25°C to -15°C. Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to homogenize solutions thoroughly before use and during reaction setup. Do not vortex the polymerase. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

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## Kit Contents

| Kit | Kit Code | Description | Component Volumes | |
|---|---|---|---|---|
| | | | 500 µL (2.5 kU) | 1000 µL (5 kU) |
| Taq DNA Polymerase (5 U/µL) | **7K0074-500UL**<br>**7K0074-1ML** | Taq DNA Polymerase (5 U/µL) | 500 µL | 1000 µL |

For larger volumes, higher concentrations, and custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

## Recommended Reaction Setup

| Component | Final Concentration | Volume (per 25 µL reaction) |
|---|---|---|
| Buffer (10X)[1,2] | 1X | 2.5 µL |
| $MgCl_2$ (25 mM)[1,3] | 2 mM | Variable |
| Each primer 10 µM (Fwd/Rev) | 0.2 µM | 0.5 µL |
| Enzyme (5 U/µL)[4] | 0.03 – 0.5 U/µL | Variable |
| dNTPs (10 mM each) | 0.2 mM | 0.5 µL |
| Template DNA[5] | Genomic 1 – 50 ng<br>Plasmid and viral 1 pg – 1 ng | Variable |
| PCR grade water | – | Up to 25 µL |

[1]Buffer (10X) and $MgCl_2$ are not supplied with Watchmaker Taq DNA Polymerase Kits.

[2]Watchmaker Taq DNA Polymerase performs well in a range of buffers. The recommended 10X buffer composition is a standard PCR buffer and will need to be optimized for specific applications.

[3]2 mM is the suggested final $MgCl_2$ concentration. $Mg^{2+}$ concentrations can alter reaction performance and may need to be optimized. A scouting range between 1 – 6 mM $MgCl_2$ is recommended. Multiplex and probe-based applications will benefit from increased $MgCl_2$ concentration.

[4]Enzyme concentration is an important parameter affecting performance and the optimal concentration will vary depending on the application. Optimizing the enzyme concentration is recommended. A good general purpose starting point is 0.05 U/µL. Use higher concentrations of polymerase, up to 0.5 U/µL, in reactions with high concentrations of inhibitors, for fast PCR, or when no amplification is observed. Use lower concentrations of polymerase if non-specific amplification is observed.

[5]Template DNA input is assay dependent and will have to be optimized.

**Note:** reverse transcriptases are usually inhibitory to PCR enzymes and 1-step qRT-PCR assays may benefit from adding higher concentrations of polymerase.

## Recommended Cycling Protocol[1]

| Step | Temperature (°C) | Time (sec) | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 60 | 1 |
| Denaturation | 95 | 15 | 25 – 35[4] |
| Annealing[2] | $T_m$~($T_m$-5) | 15 | |
| Extension[3] | 72 | 30 | |
| Final extension | 72 | 60 | 1 |
| – | 12 | Hold | – |

[1]Increased PCR specificity can be achieved by performing the reaction setup on ice and inserting reaction tubes into a thermocycler preheated to the denaturing temperature of 95°C

[2]The optimal annealing temperature should be determined empirically in an annealing temperature gradient experiment.

[3]The general recommendation for *Taq* DNA polymerase is 1 min/kb with a minimum of 30 sec for amplicons shorter than 500 bp. Reactions requiring fast PCR can be optimized by increasing the enzyme concentration, shortening the length of the extension step and by performing two-step PCR (combining annealing/extension at the temperature of annealing).

[4]Cycle number will vary depending on starting DNA input.

## Revision History

| Version | Description | Date |
|---|---|---|
| 1.0 | • First protocol release | 12/2023 |

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