# Glycerol free TEMPase Hot Start DNA Polymerase

With 10x Key Buffer (15 mM $MgCl_2$)

**Conc.: 5 units/µl**

7 312056 578147

## Cat. No.: 733-2556 (2500 Units)

| - | Size Units | TEMPase Polymerase, Glycerol free, 5 U/µl | 10x Key Buffer, 15 mM $MgCl_2$ | $MgCl_2$ 25 mM |
|---|---|---|---|---|
| **ID No. Cap colour** | - | **5101700 White** | **5100950 White** | **5575801 Yellow** |
| **733-2555** | 500 | 100 µl | 1.5 ml | 1.5 ml |
| **733-2556** | 2500 | 5 x 100 µl | 5 x 1.5 ml | 5 x 1.5 ml |
| **733-2557 sample** | 50 | 1 x 10 µl | 1.5 ml | 1.5 ml |

**Store at -20 °C**.

## General Description

TEMPase Hot Start DNA Polymerase is a modified form of VWR Taq DNA Polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity and greater yields when compared to standard DNA polymerases. Once the reaction reaches optimal activating temperature, the chemical moiety is cleaved during a 15-minute heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

## Key Features

- Convenient reaction set up at room temperature
- TEMPase Hot Start enzyme for increased sensitivity, specificity and product yield
- Designed to diminish the formation of non-specific product
- Detection of low copy number targets
- Freeze drying and automation processes

### TEMPase Hot Start Storage Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween® 20, 0.5 % NP40.

### 10x Key Buffer

Tris-HCl, pH 8.5 $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1 % Tween® 20.

### 25 mM $MgCl_2$

25 mM $MgCl_2$ in PCR grade water

### Quality Control

Each lot of TEMPase DNA Polymerase is tested for contaminating activities, with no trace of endonuclease activity, nicking activity or exonuclease activity.

### Unit Definition

One unit is defined as the amount of polymerase that incorporates 10 nmoles of dNTPs into acid-precipitable form of DNA in 30 minutes at 72°C under standard assay conditions.

## Protocol

This protocol serves as a guideline for PCR. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

### Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- 15 mM $MgCl_2$ is present in the 10x Key Buffer. The 1x concentration is 1.5 mM $MgCl_2$.
- In some applications, more than 1.5 mM $MgCl_2$ is needed for the best results. For this reason, 25 mM $MgCl_2$ is included in the kit. Table 1 provides the volume of 25 mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

Table 1. $MgCl_2$ concentration in a 50 µl reaction

| Final $MgCl_2$ conc. in reaction (mM) | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
|---|---|---|---|---|---|---|---|
| Additional volume of 25 mM $MgCl_2$ per reaction (µl): | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

1. Thaw Glycerol Free TEMPase, 10x Key Buffer, dNTP mix and primer solutions. **It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts.**

   **Important:** Spin the vials briefly before use.

2. Prepare a master mix according to Table 2.

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master mix up and down a few times.

4. Program the thermal cycler according to the manufacturer's instructions. **Each program must start with an initial heat activation step at 95 °C for 15 minutes.**

   For maximum yield and specificity, temperatures and cycling times should be optimized for each new target template or primer pair.

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Table 2. Reaction components (master mix and template DNA)

| Component | Vol./reaction* | Final concentration* |
|---|---|---|
| 10x Buffer | 5 µl | 1x |
| 25 mM $MgCl_2$ | 0 µl (0 – 7 µl) | 1.5 mM (0.5 – 5 mM) |
| dNTP mix (10 mM each) | 1 µl | 0.2 mM of each dNTP |
| Primer A (10 µM) | 1 µl (0.5 – 5 µl) | 0.2 µM (0.1 – 1.0 µM) |
| Primer B (10 µM) | 1 µl (0.5 – 5 µl) | 0.2 µM (0.1 – 1.0 µM) |
| TEMPase Hot Start DNA Polymerase | 0.4 µl (0.2 – 1 µl) | 2 units (1 – 5 units) |
| PCR-grade $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 50 ng (10 – 500 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>bacterial DNA: 5 ng (1 – 10 ng) |
| **TOTAL volume** | 50 µl | - |

* Suggested starting conditions; theoretically used conditions in brackets

5. Place the tubes in the thermal cycler and start the reaction.

Three-step PCR Program

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| 1 | 15 minutes[a] | 95 °C |
| 25-35 | 20 – 30 seconds[b]<br>20 – 40 seconds[c]<br>30 seconds[d] | 95 °C<br>50 – 65 °C<br>72 °C |
| 1 | 5 minutes[e] | 72 °C |

[a] For activation of the TEMPase Hot Start DNA Polymerase.

[b] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c] Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the $T_m$ of the primers used.

[d] Extension/elongation step: TEMPase Hot Start DNA Polymerase has its optimal activity at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the TEMPase Hot Start DNA polymerase will polymerize a thousand bases per minute.

[e] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

## Related Products

| Description | Cat. No. |
|---|---|
| **VWR Taq DNA Polymerase** | |
| VWR Glycerol Free Taq DNA Polymerase (1000 units)<br>with 10x Key Buffer<br>with 10x Extra Buffer | **733-1817** |
| VWR Taq DNA Polymerase (500 units)<br>with 10x Key Buffer<br>with 10x Extra Buffer | **733-1301** |
| VWR Taq DNA Polymerase (500 units)<br>with 10x Extra Buffer ($Mg^{2+}$-free and Triton-free) | **733-1304** |
| VWR Taq DNA Polymerase (1000 units)<br>with 10x Key Buffer (Tween-free)<br>with 10x Extra Buffer (Triton-free) | **733-1307** |
| VWR Taq DNA Polymerase (500 units)<br>with 10x Key Buffer ($Mg^{2+}$-free) | **733-1311** |
| VWR Red Taq DNA Polymerase (2500 units)<br>with 10x Key Buffer<br>with 10x Extra Buffer | **733-1323** |
| **VWR Taq DNA Polymerase Master Mix*** | |
| VWR Taq DNA Pol. 1.1x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-1314** |
| VWR Taq DNA Pol. 2x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-1316** |
| VWR Red Taq DNA Pol. 1.1x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-1318** |
| VWR Red Taq DNA Pol. 2x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-1320** |
| **VWR TEMPase Hot Start DNA Polymerase** | |
| VWR TEMPase Hot Start DNA Polymerase (500 units)<br>with 10x Key Buffer<br>with 10x Combination Buffer | **733-1331** |
| VWR Glycerol Free TEMPase DNA Polymerase (500 units)<br>with 10x Key Buffer | **733-2555** |
| **VWR TEMPase Hot Start Master Mix**** | |
| VWR TEMPase Hot Start 2x Master Mix K, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-2582** |
| VWR Blue TEMPase Hot Start 2x Master Mix K, 1.5 mM $MgCl_2$ final conc. (2500 reactions) | **733-2585** |
| **VWR Multiplex Master Mix** | |
| VWR Multiplex 2x Master Mix, (2500 reactions) | **733-2569** |
| **VWR High Fidelity DNA Polymerase** | |
| VWR AccuPOL DNA Polymerase (250 units)<br>with 10x Key Buffer | **733-1324** |
| VWR AccuPOL DNA Polymerase (250 units)<br>with 10x Key Buffer ($Mg^{2+}$ free and Tween free) | **733-1328** |
| **VWR PCR accessories** | |
| Betaine Enhancer Solution 5 M, 5 ml (500 reactions) | **733-1361** |
| dNTP Mix, 40 mM (10 mM of each dATP, dCTP, dGTP & dTTP) (40 µmol, 2x 500 µl) | **733-1363** |
| dNTP Set, 100 mM of each dATP, dCTP, dGTP & dTTP (4x 25 µmol, 4x 250 µl) | **733-1364** |
| VWR PCR grade water (6 x 5 ml) | **733-2573** |
| VWR Loading Buffer Red (5 x 1 ml) – available in 4 different colors | **733-2574** |

*Also available with 2 mM $MgCl_2$
**Also available as 2x Master Mix C based on Combination Buffer



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