

## VWR Glycerol Free Taq DNA Polymerase

Conc.: 5 units/ $\mu$ l



Cat. No.: 733-1999 (5000 Units)

	Size Units	Taq DNA Polymerase Glycerol free 5 U/ $\mu$ l	MgCl <sub>2</sub> 25 mM
ID No. Cap colour	-	5101500 Black	5575801 Yellow
733-1999	5000	10 x 100 $\mu$ l	3 x 5 ml
733-1818 Sample	50	1 x 10 $\mu$ l	1 x 1.5 ml

Store at -20 °C. Reagent for *in vitro* laboratory use only

### General Description

VWR Glycerol Free Taq DNA Polymerase is a thermostable recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. The enzyme is isolated from *Thermus aquaticus* and has a molecular weight of approximately 94 kDa.

VWR Glycerol Free Taq DNA Polymerase has both a 5'→3' DNA polymerase and a 5'→3' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity (no proofreading ability). VWR Glycerol Free Taq DNA Polymerase leaves an A' overhang, which makes the enzyme ideal for TA cloning. The glycerol free formulation makes it especially well-suited for freeze drying and automatization.

### Storage and Dilution Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween® 20, 0.5 % NP40.

### Quality Control

Each lot of VWR Glycerol Free Taq 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 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<sub>2</sub> is present in common VWR 10x buffers. The 1x concentration is 1.5 mM MgCl<sub>2</sub>.
- In some applications, more than 1.5 mM MgCl<sub>2</sub> is needed for best results. For this reason, 25 mM MgCl<sub>2</sub> is included in the kit. Table 1 provides the volume of 25 mM MgCl<sub>2</sub> to be added to the master mix if a higher MgCl<sub>2</sub> concentration is required.

Table 1. MgCl<sub>2</sub> concentration in a 50  $\mu$ l reaction

Final MgCl <sub>2</sub> conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl <sub>2</sub> per reaction ( $\mu$ l):	0	1	2	3	4	5	6

- Thaw 10x 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.** Keep all components on ice.
- Prepare a master mix according to Table 2. The master mix typically contains all the components needed for extension except the template DNA.

Table 2. Reaction components: Master mix and template DNA

Component	Vol./reaction*	Final concentration*
10x Buffer	5 $\mu$ l	1x
25 mM MgCl <sub>2</sub>	0 $\mu$ l (0 – 7 $\mu$ l)	1.5 mM (0.5 – 5 mM)
dNTP mix (12.5 mM each)	0.8 $\mu$ l	0.2 mM of each dNTP
Primer A (10 $\mu$ M)	1 $\mu$ l (0.5 – 5 $\mu$ l)	0.2 $\mu$ M (0.1 – 1.0 $\mu$ M)
Primer B (10 $\mu$ M)	1 $\mu$ l (0.5 – 5 $\mu$ l)	0.2 $\mu$ M (0.1 – 1.0 $\mu$ M)
Taq DNA Pol.	0.2 $\mu$ l (0.2 – 1 $\mu$ l)	1 unit (1 – 5 units)
PCR-grade H <sub>2</sub> O	X $\mu$ l	-
Template DNA	X $\mu$ l	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
<b>TOTAL volume</b>	50 $\mu$ l	-

\* Suggested starting conditions; theoretically used conditions in brackets

- 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.
- Add template DNA to the individual tubes containing the master mix.
- Program the thermal cycler according to the manufacturer's instructions.

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

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

### Three-step PCR Program

Cycles	Duration of cycle	Temperature
1	2 minutes <sup>a</sup>	95 °C
25 – 35	20 – 30 seconds <sup>b</sup> 20 – 40 seconds <sup>c</sup> 30 seconds <sup>d</sup>	95 °C 50 – 65 °C 72 °C
1	5 minutes <sup>e</sup>	72 °C

- a. Initial denaturation step (optional).
- 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: Taq 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 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.
<b>VWR Taq DNA Polymerase</b>	
VWR Glycerol Free Taq DNA Polymerase (1000 units) with 10x Key Buffer with 10x Extra Buffer	733-1817
VWR Taq DNA Polymerase (500 units) with 10x Key Buffer with 10x Extra Buffer	733-1301
VWR Taq DNA Polymerase (500 units) with 10x Extra Buffer ( $Mg^{2+}$ -free and Triton-free)	733-1304
VWR Taq DNA Polymerase (1000 units) with 10x Key Buffer (Tween-free) with 10x Extra Buffer (Triton-free)	733-1307
VWR Taq DNA Polymerase (500 units) with 10x Key Buffer ( $Mg^{2+}$ -free)	733-1311
VWR Red Taq DNA Polymerase (2500 units) with 10x Key Buffer with 10x Extra Buffer	733-1323
<b>VWR Taq DNA Polymerase Master Mix*</b>	
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
<b>VWR TEMPase Hot Start DNA Polymerase</b>	
VWR TEMPase Hot Start DNA Polymerase (500 units) with 10x Key Buffer with 10x Combination Buffer	733-1331
VWR Glycerol Free TEMPase DNA Polymerase (500 units) with 10x Key Buffer	733-2555
<b>VWR TEMPase Hot Start Master Mix**</b>	
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
<b>VWR Multiplex Master Mix</b>	
VWR Multiplex 2x Master Mix, (2500 reactions)	733-2569
<b>VWR High Fidelity DNA Polymerase</b>	
VWR AccuPOL DNA Polymerase (250 units) with 10x Key Buffer	733-1324
VWR AccuPOL DNA Polymerase (250 units) with 10x Key Buffer ( $Mg^{2+}$ free and Tween free)	733-1328
<b>VWR PCR accessories</b>	
Betaine Enhancer Solution 5 M, 5 ml (500 reactions)	733-1361
dNTP Mix, 40 mM (10 mM of each dATP, dCTP, dGTP & dTTP) (40 $\mu$ mol, 2x 500 $\mu$ l)	733-1363
dNTP Set, 100 mM of each dATP, dCTP, dGTP & dTTP (4x 25 $\mu$ mol, 4x 250 $\mu$ l)	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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