

VWR Red Tag DNA Polymerase

With 10x Key Buffer (15 mM MgCl₂) With 10x Extra Buffer (15 mM MgCl₂)

Conc.: 5 units/µl

7"312056" 230540

Cat. No.: 733-1834 (10000 Units)

-	Size Units	Taq Red DNA Polymerase 5 U/µl	10x Key Buffer, 15 r	10x Extra	MgCl₂ 25 mM
ID No. Cap colour	-	5101750 Red	5100950 White*	5100510 Blue*	5575801 Yellow*
733-2408	500	100 µl	1.5 ml	1.5 ml	1.5 ml
733-1323	2500	5 x 100 µl	5 x 1.5 ml	5 x 1.5 ml	5 x 1.5 ml
733-1834	10000	3 x 667 µl	6 x 5.0 ml	6 x 5.0 ml	6 x 5.0 ml
733-1835 sample	50	10 µl	1.5 ml	1.5 ml	1.5 ml

^{*} clear for 5 ml tubes

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

General Description

VWR Red Taq DNA Polymerase is a thermostable recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. VWR Red Taq DNA Polymerase contains a red dye which provides easy and quick identification of reactions to which enzyme was added and allows confirmation of complete mixing. The inert dye has no effect on downstream processes. VWR Red Taq DNA Polymerase is added directly to the reaction mix and is used in the same manner as standard Taq DNA Polymerase.

VWR Red Taq DNA Polymerase has both a 5' \rightarrow 3' DNA polymerase and a 5' \rightarrow 3' exonuclease activity. The enzyme lacks a 3' \rightarrow 5' exonuclease activity. VWR Red Taq DNA Polymerase leaves an A' overhang, which makes the enzyme ideal for TA cloning.

10x Key Buffer

Tris-HCl pH 8.5, (NH₄)₂SO₄, 15 mM MgCl₂, 1 % Tween[®] 20.

10x Extra Buffer

Tris-HCl pH 8.5, KCl, 15 mM MgCl₂, 1 % Triton X-100.

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, inert red dye, 0.5 % Tween $^{\circ}$ 20, 0.5 % NP40, 50 % glycerol.

Quality Control

Each lot of VWR 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 MgCl₂ is present in the 10x buffers. The 1x concentration is 1.5 mM MgCl₂.
- In some applications, more than 1.5 mM MgCl₂ is needed for best results. For this reason, 25 mM MgCl₂ is included in the kit. Table 1 provides the volume of 25 mM MgCl₂ to add to the master mix if a higher MgCl₂ concentration is required.

Table 1. MgCl₂ 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 (µI):	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. The polymerase is provided in glycerol and does not need thawing. Keep it at -20 °C at all times.
- Prepare a master mix according to Table 2. The master mix typically contains all the components needed for extension except the template DNA.
- 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.
- 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 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 μΙ (0 – 7 μΙ)	1.5 mM (0.5 – 5 mM)
dNTP mix (12.5 mM each)	0.8 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)
Taq DNA Pol.	0.2 µl (0.2 – 1 µl)	1 unit (1 – 5 units)
PCR-grade H₂O	Xμl	-
Template DNA	ΧμΙ	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	50 µl	-

^{*} Suggested starting conditions; theoretically used conditions in brackets

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

Three-step PCR Program

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

^{a.} Initial denaturation step (optional).

Related Products

Description	Cat. No.			
VWR Taq DNA Polymerase				
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			
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) with 10x Key Buffer with 10x Combination Buffer	733-1331			
VWR Glycerol Free TEMPase DNA Polymerase (500 units) 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) with 10x Key Buffer	733-1324			
VWR AccuPOL DNA Polymerase (250 units) 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 μ mol, 2x 500 μ l)	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 *Also available with 2 mM MgCla	733-2574			

^{*}Also available with 2 mM MgCl₂

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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.

 $^{^{\}rm c}$. 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 singlestranded DNA is fully extended.

^{**}Also available as 2x Master Mix C based on Combination Buffer