VWR Taq DNA Polymerase

With 10x Key Buffer (Mg^{2+} free)

Conc.: 5 units/µl

Cat. No.: 733-1311 (500 Units)

-	Size Units	Taq DNA Polymerase 5 U/µl	10x Key Buffer, Mg^{2+} free	$MgCl_2$ 25 mM
ID No. Cap colour	**-**	**5101600 Purple**	**5101000 Green***	**5575801 Yellow***
733-1311	500	100 µl	1.5 ml	1.5 ml
733-1312	1000	2 x 100 µl	2 x 1.5 ml	2 x 1.5 ml
733-1313	2500	5 x 100 µl	5 x 1.5 ml	5 x 1.5 ml
733-2009	10000	3 x 667 µl	6 x 5 ml	6 x 5 ml
733-1825 sample	50	1 x 10 µl	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 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 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 Taq DNA Polymerase leaves an A′ overhang, which makes the enzyme ideal for TA cloning.

10x Key Buffer, Mg^{2+} free

Tris-HCl pH 8.5, $(NH_4)_2SO_4$ and 1 % Tween® 20.

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, 50 % glycerol.

Quality Control

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

Suggested Protocol Using VWR Taq DNA Polymerase

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.
- When using Mg^{2+} free buffers, the addition of $MgCl_2$ to the reaction is imperative because Mg^{2+} is required for polymerase activity. Use 25 mM $MgCl_2$ to adjust the Mg^{2+} concentration according to Table 1.

Table 1. Additional volume (µl) of $MgCl_2$ per 50 µl reaction

Final $MgCl_2$ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$ (µl)	1	2	3	4	5	6	7	8	9

1. 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.

2. 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 µl	1x
25 mM $MgCl_2$	3 µl (1 – 10 µl)	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.	0.4 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) 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

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.

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4. Add template DNA to the individual tubes containing the master mix.

5. 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.

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] 20 – 40 seconds[c] 30 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	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.
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 (Tween 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., 1.1 x Master Mix, 1.5 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1314**
VWR Taq DNA Pol., 1.1 x Master Mix, 2 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1315**
VWR Taq DNA Pol., 2 x Master Mix, 1.5 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1316**
VWR Taq DNA Pol., 2 x Master Mix, 2 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1317**
VWR Red Taq DNA Pol., 1.1 x Master Mix, 1.5 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1318**
VWR Red Taq DNA Pol., 1.1 x Master Mix, 2 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1319**
VWR Red Taq DNA Pol., 2 x Master Mix, 1.5 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1320**
VWR Red Taq DNA Pol., 2 x Master Mix, 2 mM $MgCl_2$ (final concentration) (2500 reactions)	**733-1321**
High Fidelity DNA Polymerase:	
AccuPOL DNA Polymerase (250 units) with 10x Key Buffer	**733-1324**
AccuPOL DNA Polymerase (250 units) with 10x Key Buffer (Mg^{2+} free and Tween free)	**733-1328**
Enhancer and dNTP:	
Betaine Enhancer Solution (5M), 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 250µl)	**733-1364**

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