Taq DNA Polymerase

10X Ammonium Buffer
10X Standard Buffer
5 units/µl

Cat. No.	Units	10X Ammonium Buffer	10X Standard Buffer	$MgCl_2$ 25 mM
AO114103	500	1.5 mL	1.5 mL	1.5 mL
AO114104	1,000	2 x 1.5 mL	2 x 1.5 mL	2 x 1.5 mL
AO114106	2,500	4 x 1.5 mL	4 x 1.5 mL	4 x 1.5 mL
AO114107	5,000	3 x 5.0 mL	3 x 5.0 mL	3 x 5.0 mL
AO114108	10,000	6 x 5.0 mL	6 x 5.0 mL	6 x 5.0 mL

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

General Description

AS ONE 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.

AS ONE 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). Taq DNA Polymerase leaves an A′ overhang, which makes the enzyme ideal for TA cloning.

10X Ammonium Buffer

Tris-HCl pH 8.5, (NH4)2S04, 15mM $MgCl_2$, 1% Tween 20®

10X Standard Buffer

Tris-HCl pH 8.5, KCl, 15mM $MgCl_2$, 1% Triton X-100

Unit Definition

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

Storage Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20®, 0.5% NP40, 50% glycerol

Unopened enzyme is stable at -20°C for 2 years after production date.

Quality Control

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

Suggested Protocol using Taq DNA Polymerase

This protocol serves as a guideline only. Optimal reaction conditions may vary and must be individually determined.

- In some applications, more than 1.5mM $MgCl_2$ is needed for the best results. For this reason, 25 mM $MgCl_2$ is included with the kit.

1. Thaw desired 10X Buffer, dNTP mix, and primer solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts**.

2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

Table 1. ***Reaction components (master mix & template DNA)***

Component	Vol./reaction	Final Conc.
10X Buffer	5 µL	1X
25 mM $MgCl_2$	0 µL (0-7 µL)	1.5 mM (1.5–5 mM)
dNTP mix (12.5 mM of each)	0.8 µL	0.2 mM of each dNTP
Primer A	1 µL (0.5-5 µL)	0.2 µM (0.1–1.0 µM)
Primer B	1 µL (0.5-5 µL)	0.2 µM (0.1–1.0 µM)
Taq DNA Polymerase	0.6 µL (0.2–1 µL)	3 units (1–5 units)
Template DNA	Variable	Variable
Distilled Water	Variable	- - - -
TOTAL volume	50 µL	- - - -

Table 2. ***$MgCl_2$ 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 rxn (µL):	0	1	2	3	4	5	6

Table 2 provides the volume of 25 mM $MgCl_2$ to add to the master mix if a certain $MgCl_2$ concentration is required.

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. 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 template target or primer pair.

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

Three- step PCR Program:

Cycles	Duration of Cycles	Temperature
1	2-5 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, but recommended for genomic DNA

[b] Denaturation step: Heating the DNA template disrupts the hydrogen bonds yielding ssDNA.

[c] Annealing step: Primers anneal to ssDNA template. Typically annealing temperature is 3-5°C below the Tm of the primers.

[d] Extension/elongation step: Taq DNA polymerase synthesizes a new DNA strand complementary to the DNA template. Extension time depends on length of DNA fragment to be amplified. At optimum temperature the DNA polymerase will polymerize 1000 bases per minute.

[e] Final elongation step: After the last PCR cycle to ensure that any remaining ssDNA is fully extended.

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