Taq DNA Polymerase

With 10X Mg^{2+}Free Ammonium Buffer

5 units/µl

Cat. No.	Units	10X Ammonium Buffer, Mg^{2+} Free	$MgCl_2$ 25 mM
AO111203	500	1.5 mL	1.5 mL
AO111204	1,000	2 x 1.5 mL	2 x 1.5 mL
AO111206	2,500	4 x 1.5 mL	4 x 1.5 mL
AO111207	5,000	3 x 5.0 mL	3 x 5.0 mL
AO111208	10,000	6 x 5.0 mL	6 x 5.0 mL
AO111209	20,000	8 x 5.0 mL	8 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 Reaction Buffer Mg^{+2} Free

Tris-HCl pH 8.5, (NH4)2S04, 1% Tween 20®.

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 and Dilution 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.

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. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

1. Thaw 10X Mg^{+2} Free Ammonium 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.

The optimal $MgCl_2$ concentration should be determined empirically but, in most cases a concentration of 1.5 mM produces satisfactory results. Table 2 provides the volume of 25mM $MgCl_2$ to add to the master mix.

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

Component	Vol./reaction	Final Conc.
10X Mg Free Buffer	5 µL	1X
25 mM $MgCl_2$	3 µL (1-8 µL)	1.5 mM (0.5–4 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	Genomic DNA 50 ng (10-500 ng) Plasmid DNA 0.5 ng (0.1-1 ng) Bacterial DNA 5 ng (1-10 ng)
Distilled Water	Variable	- - - -
TOTAL volume	50 µL	- - - -

Table 2. ***$MgCl_2$ concentration***

Final $MgCl_2$ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Additional volume of 25 mM $MgCl_2$ per reaction (µL):	1	2	3	4	5	6	7	8

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.