High Fidelity DNA Polymerase

10X Ammonium Buffer
2.5 units/µL

Cat. No.	Units	10X Ammonium Buffer	$MgCl_2$ 25 mM
AO211103	500	1.5 mL	1.5 mL
AO211104	1,000	2 x 1.5 mL	2 x 1.5 mL
AO211106	2,500	4 x 1.5 mL	4 x 1.5 mL

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

General Description

AS ONE High Fidelity DNA Polymerase is a thermostable enzyme with proofreading ability, which can be used in primer extension reactions and other molecular biology applications. High Fidelity Taq Polymerase exhibits both 5'→3' DNA polymerase activity and 3'→5' proofreading exonuclease activity. The High Fidelity-Taq error rate of 1.1 $x10^{-6}$ gives it a 16x greater fidelity than Taq polymerase.

Optimal reaction conditions are achieved by using the 10x Ammonium buffer containing $MgCl_2$ provided with the enzyme. 25 mM $MgCl_2$ is also included separately, in case a higher $MgCl_2$ concentration is required for a specific reaction.

High Fidelity-Taq is recommended for applications which require extremely high fidelity or blunt ending.

Key Features

- Provides higher fidelity than standard Taq DNA Polymerase
- Produces blunt-ended fragments
- Processes <3 kb with extremely high fidelity

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.

10X Ammonium Reaction Buffer

Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 1% Tween® 20, 15mM $MgCl_2$

High Fidelity Taq Storage Buffer

50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, 0.1% NP40, 0.1% Tween-20.

Quality Control

Endonuclease, exonuclease and priming activities are not detected after 3 hours incubation of 1 µg of pUC19 plasmid DNA and 0.5 µg *Eco*R I digested lambda phage DNA at 72°C in the presence of 40 units of High Fidelity DNA Polymerase.

Suggested Protocol using High Fidelity Taq Polymerase

This protocol serves as a guideline. Optimal reaction conditions must be individually determined.

1. Thaw 10X 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, as provided in the 1X Ammonium Buffer, will produce satisfactory results. Table 2 provides the volume of 25 mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

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

Component	Vol./reaction	Final Conc.
10X Ammonium 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	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
High Fidelity Taq Polymerase	1 µL	2.5 units/reaction
Distilled Water	Variable	- - - -
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)
TOTAL volume	50 µL	- - - -

Table 2. ***$MgCl_2$ concentration in a 50µl 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 (µL):	0	1	2	3	4	5	6

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 (0.1–0.5 µg/reaction) to the individual tubes containing the master mix.

5. Program the thermal cycler according to the manufacturer's instructions. High Fidelity Taq is a proofreading enzyme and requires an extension time of 1-2 min./kb. 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	1-2 minutes[a]	95°C
25-35	30-60 seconds[b] 30 seconds[c] 1-4 minutes[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.