Hot Start Taq DNA Polymerase

5 units/µl

Cat. No.	Size Units	10X Ammonium Buffer ($MgCl_2$ 15 mM)	10X Combination Buffer ($MgCl_2$ 15 mM)	$MgCl_2$ 25 mM
AO225103	500	1.5 mL	1.5 mL	1.5 mL
AO225104	1,000	2 x 1.5 mL	2 x 1.5 mL	2x 1.5 mL
AO225106	2,500	4 x 1.5 mL	4 x 1.5 mL	4x 1.5 mL
AO225107	5,000	3 x 5.0 mL	3 x 5.0 mL	3 x 5.0 mL
AO225108	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 Hot Start Taq DNA Polymerase is a modified form of AS ONE Taq DNA Polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity and greater yields when compared to standard DNA polymerases.

Once the reaction reaches optimal activating temperature, the chemical moiety is cleaved during a 15 minute heat activation step, releasing the active HS Taq DNA Polymerase into the reaction.

10X Ammonium Buffer

Tris-HCl, pH 8.5 $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween 20®.

10X Combination Buffer

Tris-HCl pH 8.7, KCl, $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween 20®.

HS Taq Storage Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20®, 0.5% NP40, 50% glycerol.

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.

Quality Control

HS Taq Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity, exonuclease or priming activity.

Suggested Protocol using HS Taq DNA Polymerase

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

- 15 mM $MgCl_2$ is present in the 10X Buffers. However, in some applications, more than 1.5 mM $MgCl_2$ is needed. For this reason, 25 mM $MgCl_2$ is included with the kit. Table 2 provides the volume of 25 mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

1. Thaw 10X Buffer, dNTP mix, 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 (0-6.5 µL)	1.5 mM (0.5-5 mM)
dNTP mix (12.5 mM each)	0.8 µL	0.2 mM each
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)
HS Taq DNA Polymerase	0.6 µL (0.2-1 µl)	3 units (1-5 units)
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 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 (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 to the individual tubes containing the master mix.

5. Program the thermal cycler according to the manufacturer's instructions. **Each program must start with an initial heat activation step at 95°C for 15 minutes.** 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.