GC-rich Hot Start Taq 2x Master Mix II

Cat. No.	Size Reactions	GC HS Taq 2x Master Mix II, 1.5 mM $MgCl_2$
AO332703	500	10 x 1.25 mL
AO332704	1,000	20 x 1.25 mL
AO332706	2,500	50 x 1.25 mL
AO332707	5,000	25 x 5 mL
AO332708	10,000	28 x 9 ml
AO332709	20,000	1 x 500 ml

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

Key Features

- For amplification of DNA targets with high GC content
- Convenient reaction set-up at room temperature
- High specificity, sensitivity and product yield
- Detection of low abundance targets
- Diminished formation of non-specific product

General Description

AS ONE GC-rich Hot Start Taq 2x Master Mix II is an all-in-one 2x master mix containing Hot Start DNA polymerase, GC Buffer II, dNTPs and $MgCl_2$. Simply mix GC HS 2x Master Mix II with primers, template and water and you are ready to carry out successful primer extensions.

HS 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, increased sensitivity and greater yields when compared to standard DNA polymerases.

Master Mix II: Combination Buffer

Combination Buffer is a proprietary mixture of K^+ and NH_4^+. This buffer combines high specificity with good product yield and high tolerance to optimization of primer annealing temperatures and Mg^{2+} concentrations due to its balanced ammonium-potassium formulation.

Composition of GC HS Taq 2x Master Mix II

0.2 units/µl HS DNA Polymerase
Tris-HCl pH 8.7, KCl, $(NH_4)_2S0_4$, 3 mM $MgCl_2$, 1% Tween 20®.
0.4 mM of each dNTPs

Storage and Stability

The unopened kit is stable at -20°C for 2 year after the production date.

Quality Control

HS DNA Polymerase is tested for contaminating activities, with no traces 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

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

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Working on ice is not required.

- The final $MgCl_2$ concentration of this 2x Master Mix is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ to adjust the Mg^{2+} concentration according to table 1.

Table 1. Additional volume (*µL*) of MgCl2 *per 50 µL reaction*

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	0	1	2	3	4	5	6

1. Thaw the Master Mix and primer solutions. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**.

2. Prepare the reaction mix. Table 2 shows the reaction set up for a final volume of 50 µl. If desired, the reaction size may be scaled down. Use 10 µl of GC HSTaq 2x Master Mix I in a final volume of 20 µl.

Table 2. ***Reaction mix and template DNA***

Component	Vol./reaction*	Final Concentration*
2x Master Mix	25 µL	1X
25 mM $MgCl_2$	0 µL (0-7 µL)	1.5 mM (1.5-5 mM)
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)
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.

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

5. Program the thermal cycler according to the manufacturer's instructions. For maximum yield and specificity, tempeatures and cycling times should be optimized for eacyh new template tafet or primer pair.

6. Each program must start with an initial heat activation step at 95°C for 15 minutes.

Three-step PCR program

Cycles	Duration of cycle	Temperature
1	15 minutes [a]	95°C
25-35	20-30 seconds[b] 20-40 seconds[c] 30 seconds[d]	95°C 55-60°C[d] 72°C
1	5 minutes[e]	72°C

[a] For activation of the hot start enzyme

[b] Denaturation time may vary between thermocyclers. This step causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c] Annealing step allows primers to anneal 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 - HS Taq has its optimum activity temperature 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 to ensure that any remaining single-stranded DNA is fully extended.