ClearLine®

15 MIN HOT START

HOT DISCO TAQ 2X MASTER MIX A

Ammonium Buffer Based, 1.5 mM $MgCl_2$ final concentration

MADE IN DENMARK

Cat. No.: 257684
2500 Reactions

-	**Hot Disco Taq 2x Master Mix A, Ammonium Buffer Based, 1.5 mM $MgCl_2$**
ID No.	**CL1.250-0028**
Cap colour	**Red**
Content	50 x 1.25 ml

Key Features

Hot Disco Taq 2x Master Mix A is an all-in-one 2x master mix containing Hot Disco Taq DNA polymerase, the ammonium buffer system, dNTPs and magnesium chloride. Each reaction requires 25 µl of the 2x Master Mix. Simply add primers, template and water to a total reaction volume of 50 µl to successfully carry out primer extensions.

Hot Disco Taq DNA Polymerase is a modified form of 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.

Composition of 2x Hot Disco Taq Master Mix A

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3.0 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Hot Disco Taq DNA Polymerase
- Stabilizer

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Option: Store at +4 °C for up to 6 months.

Quality Control

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

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using Hot Disco Taq 2x Master Mix A. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

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**.

   Important: Spin vials briefly before use.

2. Prepare the reaction mix. Table 1 shows the reaction mix set up for a final volume of 50 µl.

Table 1. Reaction mix and template DNA

Component	**Vol./reaction***	**Final concentration***
Master Mix	25 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 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: 20 ng (1 – 200 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 reaction mix thoroughly and dispense appropriate volumes into reaction tubes.

4. Add template DNA to the individual tubes containing the reaction 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.** See table 2 for an example.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

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

Table 2. 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– 90 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

[a] For activation of the hot start enzyme.

[b] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c] Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the T_m (melting temperature) of the primers used.

[d] Extension/elongation step: Hot Disco Taq DNA polymerase 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 at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Notes:

- The final $MgCl_2$ concentration of this Hot Disco Taq 2x Master Mix A is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM to adjust the Mg^{2+} concentration according to table 3.

Table 3. Additional volume (µl) of $MgCl_2$ 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

- For longer DNA targets more DNA polymerase could be added to the PCR master mix.

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Other product sizes, combinations and customized solutions are available. Please look at www.dutscher.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

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