# HOT DISCO TAQ

**With 10x Ammonium Buffer (15 mM $MgCl_2$)**

**Concentration: 5 units/µl**

15 MIN HOT START

## Cat. No.: 257663

**2500 Units**

| - | Hot Disco Taq 5 U/µl | 10x Ammonium Buffer, 15 mM $MgCl_2$ | $MgCl_2$ 25 mM |
|---|---|---|---|
| **ID No.** | **CL0.100-0022** | **CL1.500-0017** | **CL1.500-0047** |
| **Cap colour** | **Red** | **White** | **Clear** |
| **Contet** | 5 x 100 µl | 5 x 1.5 ml | 5 x 1.5 ml |

## Key Features

Hot Disco Taq is a modified form of ClearLine Disco Taq. 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. Once the reaction reaches optimal activating temperature, the chemical moiety is cleaved during a 15 minutes heat activation step, releasing the active Hot Disco Taq into the reaction.

## Kit Components

### Hot Disco Taq in Storage Buffer

5 U/µl Hot Disco Taq, 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 50% glycerol.

### 10x Ammonium Buffer

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

Ammonium Buffer gives a superior amplification signal (high yield) in most primer-template systems. Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration and the annealing temperature.

### $MgCl_2$

25 mM $MgCl_2$ in PCR grade water.

### 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

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

### Unit Definition

One unit is defined as the amount of polymerase that incorporates 10 nmol of dNTPs into acid-precipitable DNA in 30 minutes at 72 °C under standard assay conditions.

## Protocol

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

1. Thaw 10x Buffer, dNTP mix and primer solutions. **It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. The polymerase is provided in glycerol and does not need thawing.

   **Important:** Spin vials briefly before use.

2. Prepare a reaction mix according to table 1. The reaction mix typically contains all the components needed for primer extension except the template DNA.

### Table 1. Reaction mix and template DNA

| Component | Vol./reaction* | Final concentration* |
|---|---|---|
| 10x Buffer | 5 µl | 1x |
| 25 mM $MgCl_2$ | 0 µl (0 – 6 µl) | 1.5 mM (1.5 – 4.5 mM) |
| dNTP mix (10 mM each) | 1 µl | 0.2 mM of each dNTP |
| 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) |
| Hot Disco Taq DNA Pol. | 0.4 µl (0.2 – 1 µl) | 2 units (1 – 5 units) |
| PCR-grade $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 20 ng (1 – 200 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>bacterial DNA: 5 ng (1 – 10 ng) |
| **TOTAL volume** | 50 µl | - |

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, eg. 0.2 µl Hot Disco Taq instead of 0.4 µl Hot Disco Taq.

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.**
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template 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]<br>20 – 40 seconds[c]<br>30 – 90 seconds[d] | 95 °C<br>50 – 65 °C<br>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 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:

- 15 mM $MgCl_2$ is present in 10x PCR Buffer. The 1x concentration is 1.5 mM $MgCl_2$. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. For this reason, 25 mM $MgCl_2$ is included in the kit. Table 2 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

### 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.



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 Europe**

Issued 02/2023