# DISCO TAQ DNA POLYMERASE, GLYCEROL FREE

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

**Concentration: 5 units/µl**

## Cat. No.: 257781

**2500 Units**

| - | Disco Taq Glycerol Free, 5U/µl | 10x Ammonium Buffer, 15 mM $MgCl_2$ | $MgCl_2$ 25 mM |
|---|---|---|---|
| **ID No.** | **CL0.020-0019** | **CL1.500-0017** | **CL1.500-0047** |
| **Cap colour** | **Black** | **White** | **Clear** |
| **Content** | 5 x 100 µl | 5 x 1.5 ml | 5 x 1.5 ml |

## Key Features

Disco Taq Glycerol Free is a thermostable, recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. The enzyme is isolated from *Thermus aquaticus* and has a molecular weight of approximately 94 kDa. Disco Taq Glycerol Free has a 5'→3' DNA polymerase and a 5'→3' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity (no proofreading ability). Disco Taq Glycerol Free leaves an A′ overhang, which makes the enzyme ideal for TA cloning.

Disco Taq Glycerol Free is ideal for freeze drying and automation.

## Kit Components

### Disco Taq Glycerol Free in Storage Buffer

5 U/µl Taq, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20.

### 10x Ammonium Buffer

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

Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration or the annealing temperature for most primer-template systems.

### 25 mM $MgCl_2$

### 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

Disco Taq Glycerol Free 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 Disco Taq. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

1. Thaw Solutions. **It is important to thaw all solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice.

2. Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.

3. 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 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 (12.5 mM each) | 0.8 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) |
| Disco Taq. | 0.2 µl (0.2 – 1 µl) | 1 unit (1 – 5 units) |
| PCR-grade $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 50 ng (10 – 500 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. 0.1 µl Taq instead of 0.2 µl Taq.

4. 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.
5. Add template DNA to the individual tubes containing the master mix.
6. Program the thermal cycler according to the manufacturer's instructions.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
7. Place the tubes in the thermal cycler and start the reaction.

### Three-step PCR program

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| 1 | 2 – 5 minutes[a] | 95 °C |

| | | |
|---|---|---|
| 25 – 35 | 20 – 30 seconds[b]<br>20 – 40 seconds[c]<br>30 seconds[d] | 95 °C<br>50 – 65 °C<br>72 °C |
| 1 | 5 minutes[e] | 72 °C |

[a.] Initial denaturation step (optional).

[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: Disco 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 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 2. 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**

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