Thermo SCIENTIFIC

# Thermo Scientific Thermo-Start *Taq* DNA Polymerase with High Performance Buffer

**Description:** Thermo-Start™ *Taq* DNA Polymerase is a chemically modified version of ThermoPrime *Taq* DNA Polymerase. It is completely inactive at room temperature, preventing the formation and subsequent amplification of non-specific products. The enzyme requires an **activation step at 95°C for 15 minutes**.

**Enzyme Source:** *Thermus aquaticus*

**Concentration:** 5 units/µl

**Unit Definition:** One unit of enzyme is defined as the amount that will incorporate 10nmoles of dNTPs into acid insoluble material in 30 minutes at 74°C under the analysis conditions below.

**Associated Activities:** Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

**Storage Conditions:** Store Thermo-Start™ *Taq* DNA Polymerase at -20°C, in a constant temperature freezer for up to 12 months. Shipped on ice within the UK and on dry ice for international and within the US.

**Kit Contents**

| Vial | Pack Size (cap color) | | |
|---|---|---|---|
| | A | B | C |
| Thermo-Start Taq DNA Pol. | 1 x 50µl (clear) | 10 x 50µl (clear) | 100 x 50µl (clear) |
| Thermo-Start High Performance PCR Buffer | 1 x 1.25ml (red) | 10 x 1.25ml (red) | 100 x 1.25ml (red) |
| $MgCl_2$ | 1 x 1.5ml (clear) | 10 x 1.5ml (clear) | 100 x 1.5ml (clear) |

**Example of Protocol:**

Mix and spin down the solutions prior to use

| | Volume | Final Concentration 1X |
|---|---|---|
| Thermo-Start Taq DNA Pol. (5U/µl) | 0.125µl | 0.625 U |
| 10X Thermo-Start HP Buffer | 2.5µl | 1X |
| dNTP Mix (20mM) | 1µl | 0.5mM of each nucleotide |
| $MgCl_2$ (25mM) | 1.5µl* | 1.5mM* |
| Primer forward (10µM each) | 1.25µl* | 0.5µM* |
| Primer reverse (10µM each) | 1.25µl* | 0.5µM* |
| Water (PCR Grade) | variable | |
| DNA Template | 0.5 - 10µl | 0.5 - 125ng |
| Total Volume | 25 µl | |

*Scale up or down the volume and concentration as appropriate

Note: These recommendations are intended as basic guidelines. Magnesium chloride concentration and amount of enzyme should be optimized according to template and primer combination.

**Example of Program:**

| | Temp. | Time | Number of cycle |
|---|---|---|---|
| **Initial Denaturation** | **95°C** | **15 min** | **1 cycle** |
| Denaturation | 95°C | 20 sec | 30 to 40 cycles |
| Annealing | 50-65°C | 30 sec | |
| Extension** | 72°C | 60 sec | |
| Final Extension | 72°C | 5 min | 1 cycle |

**Increase length of time in proportion to size of amplicon, Taq DNA Polymerase extends at approximately 1000 bp/min.

**Incremental Activation:**

For extra stringency, the enzyme can be activated gradually during the PCR in a series of steps. The initial activation step is replaced by longer (2 minutes) denaturation steps for the first 7–8 cycles of the reaction.

**Analysis Conditions:**

| | |
|---|---|
| 25mM | TAPS, pH 9.3 (at 25°C)<br>[tris-(hydroxymethyl)-methyl-amino-propane sulfonic acid, sodium salt] |
| 50mM | KCl |
| 2mM | $MgCl_2$ |
| 1mM | β-mercaptoethanol |
| 200µM | of each: dATP, dGTP, dTTP |
| 100µM | [$\alpha^{32}$P]-dCTP (0.05 to 0.1 Ci/mmol) |
| 1.25µg/µl | activated salmon sperm DNA |

Water added to a total volume of 50µl. Incubated at 74°C for 10 minutes. The enzyme is first treated with a 15 minute activation step at 95°C. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

**Storage Buffer:**

| | |
|---|---|
| 100mM | KCl |
| 20mM | Tris-HCl, pH 9.2 (at 25°C) |
| 0.1mM | EDTA (ethylenediaminetetraacetic acid) |
| 1mM | DTT (dithiothreitol) |
| 0.5% | Tween® 20 |
| 0.5% | Nonidet® P40 |
| 50% (v/v) | Glycerol |

**Ordering Information:**

| | | |
|---|---|---|
| AB-1057/A | Thermo-Start *Taq* DNA Polymerase with High Performance Buffer | 250 units |
| AB-1057/B | Thermo-Start *Taq* DNA Polymerase with High Performance Buffer | 10 x 250 units |
| AB-1057/C | Thermo-Start *Taq* DNA Polymerase with High Performance Buffer | 100 x 250 units |

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

| **Troubleshooting:** | **Email** | **Phone** |
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