

Thermo-Start™ ReddyMix™ PCR Kit

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.**

ReddyMix™ PCR Buffer has an inert red tracker dye and a precipitant added. After thermal cycling a sample (10–30%) of the PCR mix may be loaded directly onto an agarose gel without the addition of gel loading buffer. The dye migrates between bromophenol blue and xylene cyanol at approximately 300bp, depending on agarose concentration.

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).

Kit Contents

| Vial (cap color) | Pack Size | |
|--|-----------|-------------|
| | A | B |
| Thermo-Start <i>Taq</i> DNA Pol. (clear) | 50μl | 10 x 50μl |
| Thermo-Start ReddyMix Buffer (yellow) | 1.25ml | 10 x 1.25ml |
| MgCl ₂ (clear) | 1.25ml | 10 x 1.25ml |
| dNTP Mix (brown)* | 0.5ml | 3 x 1.6ml |

*contains 5mM of each dNTP (dATP, dGTP, dCTP and dTTP)

For Research Purposes Only

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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.

Example of Protocol:

Mix and spin down the solutions prior to use

| | Volume | Final Concentration 1X |
|--|------------|--------------------------|
| Thermo-Start <i>Taq</i> DNA Pol. (5U/μl) | 0.125μl | 0.625 U |
| 10X ReddyMix™ Buffer | 2.5μl | 1X |
| dNTP Mix (20mM) | 1μl | 0.2mM 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* |
| DNA Template | 0.5 - 10μl | 0.5 - 125ng |
| Water (PCR Grade) | To 25μl* | |

*Scale up or down the volume and concentration as appropriate

Tip:

These recommendations are intended as basic guidelines. Magnesium chloride concentration and amount of enzyme should be optimized according to template and primer combination. The gel precipitant in ReddyMix™ Buffer causes a slight increase in the thermal mass of the reaction mix. In a small number of cases this may necessitate some minor re-optimization of the thermal cycler program. If this is the case we suggest increasing the temperature of the denaturation step by 1–2°C and decreasing the temperature of the annealing step by 1–2°C. Alternatively, increase the duration of each step by 5–10 seconds.

Example of Program:

| | Temp. | Time | Number of cycle |
|-----------------------------|-------------|---------------|-----------------|
| Initial Denaturation | 95°C | 15 min | 1 cycle |
| Denaturation | 95°C | 25 sec | 30 to 40 cycles |
| Annealing | 48-63°C | 35 sec | |
| Extension** | 72°C | 65 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.

Buffer composition

| | | |
|-----------------------------|-----------|--|
| Enzyme Storage and Dilution | 100mM | KCl |
| Buffer: | 20mM | Tris-HCl, pH 8.0 (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-1950/A/N | Thermo-Start™ ReddyMix™ PCR Kit | 250 units |
| AB-1950/B/N | Thermo-Start™ ReddyMix™ PCR Kit | 2500 units |

All sizes are supplied with 10X Reaction Buffer, 20mM dNTP Mix and 25mM MgCl₂.

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